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JANUARY
1921

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TABLE OF CONTENTS

FOR VOLUME XL, Number 1, January, 1921

Acanthocephala from the Eel, with one plate, by H. J. VanCleave..... 1

A Brief Study of the Range of Error in Micro-Enumeration, by W. E. Allen..... 14

Department of Methods, Reviews, Abstracts, and Briefer Articles

Remarks on the Life-history and the Scale Characters of American Mullets, by C. L. Hubbs..... 26

Spring Migration in the Crayfish, Cambarus argillicola Faxon, by H. Cummins... 28

Preparing Collections of the Mollusca for Exhibition and Study, with five figures, by F. C. Baker..... 31

Proceedings of the American Microscopical Society..... 47

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TRANSACTIONS
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ACANTHOCEPHALA FROM THE EEL[1]

BY
H. J. VAN CLEAVE

Introduction
Materials and Acknowledgments
Records of Acanthocephala infesting *Anguilla chrysypa*
Descriptions of Species
Tonaorhamphus ambiguus n. sp.
Neoechinorhynchus cylindratus (Van C., 1913)
Echinorhynchus coregoni Linkins in Van C., 1919
Echinorhynchus thecatus Linton, 1891
Uncertain Identifications of Species
Neoechinorhynchus agilis (Rud.)
Koleops anguilla Lockwood
Echinorhynchus clavaeceps Zeder
Echinorhynchus globulosus Rud.
Conclusions
Acanthocephala from *Anguilla vulgaris*
Literature Cited
Explanation of Plate

INTRODUCTION

Because of their migratory habits, eels offer some fascinating problems to investigators interested in the geographical distribution of parasitic organisms. The migrations of most other animals are between places of essentially similar surroundings and for that reason little evidence is available regarding the source of infestations borne by the wanderers. Very little is known of the definite limitations to the distribution of parasites of purely limnetic and terrestrial organisms. On the other hand most of the larger groups of parasitic organisms include species or even genera which are distinctively limited to either a marine or a fresh-water habitat. One of the most interesting and perplexing features of the parasitism of migratory organisms is the fact that they may carry throughout their range mature parasites the larval stages of which have been acquired in a very narrowly restricted locality. Because of the fairly sharp contrast

[1] Contributions from the Zoological Laboratory of the University of Illinois, No. 175.

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between the marine and the fresh-water faunas, organisms like the eel which wander from one to the other offer exceptional opportunity for the study of the influence of migration upon the parasitic fauna of the host. In such migrants it becomes possible to recognize the source of a parasitic infestation with much greater certainty than is possible when the migratory movements are between localities of approximately the same physical environment.

The investigations of Zschokke upon the parasites of salmon stand as probably the most comprehensive work in the literature upon the parasites of migratory fishes. In his studies he included observations upon individuals ascending the Rhine and upon others taken from the Baltic Sea. From the former he recorded twenty species of parasitic worms all of which were apparently acquired by the salmon before they left the ocean. The lack of parasites of limnetic origin is obviously correlated with the fact that all evidence seems to indicate that the Rhine salmon commonly refuses to take food after entering the river. In the Baltic salmon, however, Zschokke found some typically fresh-water species of parasites occurring with the ostensibly marine species. Zschokke's work has been summarized by Ward (1910:1160) in the following manner: "The Rhine salmon shelters a purely marine parasitic fauna, while the Baltic salmon reckons many limnetic forms among its parasitic guests. This remarkable condition finds explanation in the continued feeding of the latter type, even in fresh water, and the resulting enrichment of its parasitic fauna with limnetic forms when it returns to the sea."

Unfortunately, the parasites of the eels have not been investigated as thoroughly as have those of the salmon and little effort has been directed toward interpretation of the isolated observations. In the present paper attention will be confined to the Acanthocephala infesting the eel. A number of investigators have compiled lists of the parasites of the European eel, *Anguilla vulgaris*, but to the present time very little material has been available regarding the Acanthocephala of the North American species, *Anguilla chrysypa*.

Any attempt to determine the origin of the parasitic fauna of the eel must take into account both the pelagic and the limnetic periods in the life cycle of an individual. Regarding the source of infestation of the adult eel found in fresh-water, at least four possibilities must be considered:

1. The parasites may have been acquired in the marine habitat and are retained for a longer or shorter period of time after the host enters fresh-water. If the host remains in fresh-water for a period longer than the life of the individual parasites constituting the original infestation, the intestine would ultimately be freed from its parasites;

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2. Eggs or larvae of parasites acquired while the host was in the ocean when discharged into fresh-water may succeed in becoming established in the new habitat through their adaptability to entirely new primary hosts;

3. The parasites may represent an entirely new infestation of typically fresh-water species acquired after the loss of any marine species that might have been carried at the time of leaving the ocean;

4. Marine and fresh-water species may become commingled in the body of the same host individual.

A number of the earlier records of Acanthocephala from *Anguilla chrysypa* have contained apparent misidentifications of species. In some of these instances the present writer has had the opportunity of examining the materials and has found that in one instance of a reputed occurrence of a marine European species from *A. chrysypa* the specimens really belong to a typically fresh-water species probably restricted to North America. In the present paper the writer hopes to analyse the earlier records of Acanthocephala from the eel and to add a considerable bulk of original data which has accrued from the study of several important collections.

Materials and Acknowledgments

In the investigation of this problem I have had the privilege of examining specimens and of incorporating data from a number of important parasite collections. Specimens from the U. S. National Museum collected by E. Linton and by A. Hassall have been especially interesting in this connection. Through the courtesy of C. C. Adams specimens secured by H. S. Pratt and F. C. Baker in the course of investigations by the New York State College of Forestry and the U. S. Bureau of Fisheries upon the fauna of Oneida Lake, New York, have been available for study.

In all the writer has examined four species of Acanthocephala from the intestine of *Anguilla chrysypa*, of which one represents a new species described here for the first time. In attempting to make a correct disposition of the species mentioned in the earlier works the writer has been extremely fortunate in being able to examine collections which have verified surmised incorrect determinations and have made corrections in the identification possible.

For data concerning the Acanthocephala of *Anguilla vulgaris* the writer has found it necessary to utilize the published records of European investigators. In a number of instances, where records are the result of compilation from various sources, there are probably errors in the determination of the species. Through the kindness of Professor K. M. Levander of Finland, I have been permitted to study the Acanthocephala encountered in the course of his investigations upon the food and parasites of the eel.

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Records of Acanthocephala Infesting *Anguilla chrysypa*

The first reference to the occurrence of an acanthocephalan in the American eel is that given by Samuel Lockwood in 1872. In a highly entertaining but superficial manner he described a new genus and new species of acanthocephalan from a cyst in the intestine of an eel ascribing to this new form the name *Koleops anguilla*. Unfortunately his description and his figures, based upon the study of a single living specimen, are so generalized that they possess but little of scientific value. Apparently the acanthocephalan from the hog is the only other species of these worms that had ever come to his attention. Both his specific description and his generic diagnosis consist in simple enumeration of a few points of difference between his specimen and "Echinorhynchus gigas." All of these differences, with the possible exception of the poorly described proboscis, might apply equally to any one of numerous genera belonging to the family Echinorhynchidae. Consequently both the genus and the species stand as unrecognizable.

Joseph Leidy in his extensive pioneer researches on fish parasites has made no mention of ever encountering Acanthocephala in the American eel.

Edwin Linton, in various reports, has given notice of the occurrence of Acanthocephala in this host. He identified (1889 and 1901) as *Echinorhynchus agilis* specimens which I have determined (Van Cleave, 1913) as *Neoechinorhynchus cylindratus*. Other specimens (1901) he believed to belong to the European species *E. globulosus*. According to his statement these last named specimens were from the collection of the U. S. National Museum. I have examined specimens from this same collection which bear a label indicating that they were identified as *E. globulosus* by Linton and seem to be the same individuals referred to in his paper just cited. A thorough study of these specimens has demonstrated that they represent an undescribed species of the genus Tanaorhamphus which is described later in this same paper.

Data concerning the parasites of fishes for A Biological Survey of the Waters of Woods Hole and Vicinity (Sumner, Osborn, and Cole, 1913) were furnished by Linton. Under *Anguilla rostrata* (= *A. chrysypa*) but two species of Acanthocephala were mentioned, namely, "*E. clavaeceps* and *E. globulosus*." The first of these is apparently a renaming of what Linton had earlier identified as "*E. agilis*" and what I have more recently shown to be *Neoechinorhynchus cylindratus*.

In the investigation of parasites of fishes from the Illinois River eight eels were examined by the writer (Van Cleave, 1919) but no Acanthocephala were discovered. In addition the writer encountered numerous negative

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records as the result of the examination of large numbers of eels taken from salt water at Woods Hole, Mass., during the month of August.

Descriptions of Species

In the light of the present investigation four valid species of Acanthocephala are to be attributed to *Anguilla chrysypa*. Of these one species is new and two of the remaining ones are reported for the first time from this host.

Tanaorhamphus ambiguus n. sp.

Definition. With the characters of the genus. Type female 7.9 mm. long; maximum diameter 0.67 mm.; diameter of posterior region of body about 0.35 mm. Proboscis cylindrical, 0.77 mm. long and 0.19 mm. in diameter, armed with twenty longitudinal rows of about sixteen hooks each. Hooks near middle of proboscis 41 to 47μ long, with the hook at base of each row about 24μ long. Proboscis receptacle 0.96 mm. long with wall composed of a single muscular layer. Central nervous ganglion at base of proboscis receptacle. Lemnisci 2.3 mm. long, cylindrical. In the type female each lemniscus contains three giant nuclei. Subcuticular giant nuclei arranged five in mid-dorsal line of body and one in mid-ventral line.

Males have not been studied. Embryos not fully formed in specimens under observation.

Type host: *Anguilla chrysypa.*

Type female collected by Albert Hassall at Baltimore, Maryland, May 30, 1891. This specimen with others of *N. cylindratus* and *E. coregoni* was deposited in the U. S. National Museum under catalog number 6301 of the Hassall Collection. The writer has examined two additional individuals belonging to this same species from the National Museum. These bore the catalog number 6471 and Linton's identification as *E. globulosus.* Neither of these was in as good condition for study as the one selected as type. One specimen, apparently a male of this species, had at some time become dried out and for that reason an accurate determination of internal structure is impossible. Linton (1901:435) states that there are three specimens in the National Museum collection, but it is entirely probable that one specimen was entirely lost at the same time that the damage was done to the male. In the reference just cited, Linton states that this species was numerous in collections made August 7 and 28, 1899.

One of the marked peculiarities of the type of this species lies in the number of nuclei within the lemnisci. All other species of Neoechinorhynchidae from North America examined by the writer agree in possessing two giant nuclei in one lemniscus and one in the other. The lemnisci of the only other specimen of this species are entirely obscured by the develop-

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ing egg masses within the body cavity. In figure 5 the three nuclei of one lemniscus are shown while two of those in the other lemniscus occur in the region where the two organs overlap. Until additional specimens are secured for study it is impossible to determine whether this deviation from the customary number of nuclei is an individual abnormality or a character of this species.

But one other species has been described for the genus Tanaorhamphus and it is apparently very sharply restricted in its occurrence, having been found in but one host, the gizzard shad (*Dorosoma cepedianum*) from the Illinois River.

Neoechinorhynchus cylindratus (Van Cleave, 1913)
Echinorhynchus agilis of Linton 1889 and 1901
Echinorhynchus clavaeceps of Linton in Sumner, Osborn and Cole, 1913.

Definition. With the characters of the genus. Body almost cylindrical except in immature forms which have the posterior region gradually attenuated. Proboscis approximately globular, slightly broader than long (0.172 by 0.150 mm.), provided with three circles of six hooks each. Hooks of terminal circle 79 to 97μ long, each bearing a root 58μ long and 29μ wide inside the tissue of the proboscis wall. Hooks of middle circle about 37μ long, without reflexed root. Basal hooks 21 to 25 μ long, simple, thorn-like. Embryos within body cavity of gravid female 49 to 51μ long and 15 to 21μ broad.

Specimens collected by Professor Linton at Woods Hole, Mass., were identified as belonging to this species by the writer in 1913 (p. 188). Additional records of the occurrence of this species in the eel have been discovered since that time. Six juvenile specimens were taken from the intestine of an eel from Oneida Lake, New York, in the materials collected by F. C. Baker and H. S. Pratt. Three other eels from the same locality were found to harbor Acanthocephala of another species but none of *N. cylindratus*. Two individuals of this species were encountered in an eel examined by A. Hassall at Baltimore, Md., along with the type of *T. ambiguus* and two individuals of *E. coregoni*.

N. cylindratus is distinctively a fresh-water species, the development of which is unknown. Without much question this species gains entrance into the eels after they enter fresh-water. The infrequence of its occurrence in the eel and the lightness of individual cases of infestation would indicate that the eel does not serve as an important definitive host for this species of parasite.

Echinorhynchus coregoni Linkins in Van Cleave, 1919

Definition. With the characters of the genus. Proboscis cylindrical, carrying twelve to fifteen longitudinal rows of ten or eleven hooks each.

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Hooks not crowded on proboscis. Basal hooks 28 to 53μ long; those on middle of proboscis 65 to 80μ long; those near anterior tip smaller and weaker than those near middle of proboscis. Ventral hooks slightly larger and stronger than dorsal hooks. Lemnisci not longer than proboscis receptacle. Cement glands of male in a compact mass. Embryos within body cavity of gravid female 51 to 91μ long by 17 to 20μ wide, with an approximately globular prolongation of the middle membrane at each pole.

This species was originally described from the Great Lakes but present indications seem to suggest that its distribution is fairly broad. The writer has recently (1920:6) recorded its occurrence in fresh-water hosts from the arctic regions of this continent. Two individuals of this species were taken from an eel at Baltimore, Md., by Hassall. As indicated earlier in this paper this species was found associated with *N. cylindratus* and *T. ambiguus*.

Echinorhynchus thecatus Linton, 1891

Definition. With the characters of the genus. Proboscis when fully extended approximately perpendicular to main axis of the body or forming an acute angle with the axis. Proboscis usually about 1 mm. long. Proboscis receptacle long and slender, about 1.5 times the length of the proboscis. Hooks alternate in arrangement; in twelve longitudinal rows of twelve or thirteen hooks each; those at base of proboscis 41 to 53μ long; near middle of proboscis 71μ long; each hook surrounded by a conspicuous cuticular elevation which frequently completely ensheathes the basal hooks of each row. Lemnisci long and slender, about 1.5 times the length of the receptacle. Embryos within body cavity of gravid female 80 to 110μ long by 24 to 30μ broad.

This species is distinctively a member of the fresh-water group. It has been reported from fishes taken from the ocean but these hosts frequent fresh-water habitats. The general outline of the life cycle of this species has been worked out (Van Cleave, 1921) thereby offering additional evidence of its close association with the fresh-water fauna where it occurs as one of the most characteristic acanthocephalan parasites of fresh-water fishes of North America. In the collections from Oneida Lake, referred to above, this species was found in fairly large numbers in the intestine of all of the eels carrying acanthocephalan infestation. This constitutes the first record of the occurrence of *E. thecatus* from *Anguilla chrysypa*.

Uncertain Identifications

Among the earlier American workers in parasitology there was a marked tendency to ascribe to specimens of Acanthocephala found in American hosts the names of European species. Thus much of the older

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literature is replete with instances of the recorded occurrence of European species in American hosts, whereas in the cases that have been investigated carefully it has been shown that with few exceptions the acanthocephalan fauna is distinctively different on the two continents. From point of view of biology and of distribution of the individual species it is extremely desirable that these errors in determination be cited and corrected if possible. In the following section the writer has attempted to analyse some of these older records in the light of more recent investigations.

Neoechinorhynchus agilis (Rudolphi)
E. agilis Rud.

The writer has examined some of Rudolphi's type specimens (Van Cleave, 1919:246) of this species and is confident that all records of the occurrence of this species on the American continent are based upon erroneous identifications. It seems probable that *N. agilis* is rather sharply restricted in distribution to the Mediterranean region.

As indicated previously, Linton (1889 and 1901) identified under this name specimens from the eel which unquestionably belong to the species *N. cylindratus*, the commonest representative of the genus in North American fishes. Furthermore *N. agilis* is a marine species while *N. cylindratus* is definitely associated with the fresh-water habitat.

Koleops anguilla Lockwood

In the description of this genus and species not a single diagnostic character is given. Characters which are discussed are all contrasted with the conditions found in "*Echinorhynchus gigas*." Apparently this last named species is the only other species of the group with which the describer was acquainted. Supposed peculiarities of the new form are in reality common to all acanthocephalans except members of the family Gigantorhynchidae. Even the drawings are not available as a supplement to the meagre description for no detail of structure, either external or internal, is shown.

"*Echinorhynchus clavaeceps*" Zeder

This species has been considered by modern writers as a synonym of *Neoechinorhynchus rutili* (Müller) which infests fresh-water fishes of central Europe. In his later works Linton apparently uses this name to replace his earlier identifications of *E. agilis*. It is highly probable that specimens identified as *E. clavaeceps* belong in reality to the species *N. cylindratus*.

Echinorhynchus globulosus Rudolphi

Linton (1901:435) identified acanthocephalans from the eel which were deposited in the U. S. National Museum as belonging to this species.

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These same specimens have been found to belong to a previously undescribed species of the genus Tanaorhamphus which is described elsewhere in this paper as *T. ambiguus*.

Conclusions

Four valid species of Acanthocephala are known to occur in the intestine of *Anguilla chrysypa*. All four of these are distinctively American species, of which three are known to occur only in fresh-water fishes. The fourth belongs to a genus which includes but one other species and it is restricted to a single species of fresh-water host. In all of the instances that have been examined carefully there is no evidence of marine species of Acanthocephala inhabiting the intestine of *Anguilla chrysypa*. In this connection a comparison with data from records of infestation of the European eel is interesting.

Acanthocephala from *Anguilla vulgaris*

Lack of unanimity in determining the synonymy of many species of Acanthocephala infesting fishes makes direct comparisons between records of European investigators extremely difficult. Before the time of Lühe many specific names, the authors of which recognized them as synonyms of earlier workers, had come into very general usage. Thus, for instance, Rudolphi (1802:53) gave the new name *Echinorhynchus angustatus* to *Ech. lucii* O. F. Müller (1778). In spite of this obvious renaming most European workers have, until very recently, failed to recognize the priority of the name *E. lucii* and have continued to use the synonym instead of the valid name of the species. Numerous instances of similar nature are encountered in the literature dealing with the Acanthocephala of fishes. In the following paragraphs names of commonly recognized synonyms have been replaced by the valid names of the species in question.

In his "Register der Acanthocephalen und parasitischen Plattwürmer," Lühe (1911:91) has compiled a list of eighteen species of parasitic worms (exclusive of nematodes) which have been recorded from the eel in central Europe. Only six of these are recognized as strictly marine, while all seven species of the Acanthocephala included in his list are typically fresh-water forms with the possible exception of the encysted larvae of an undetermined species of the genus Corynosoma.

K. M. Levander (1909) in his admirable contribution to the knowledge of the food and parasites of the fishes of Finland has utilized the names current in the literature for the species of Acanthocephala encountered in his investigations. His data upon the parasites of *Anguilla vulgaris* include three species of Acanthocephala from this host. Since the time of the publication of his work the names *Echinorhynchus angustatus*, *E.*

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globulosus, and *E. clavaeceps* have become recognized as synonyms of *Acanthocephalus lucii* (Müller), *Ac. anguillae* (Müller), and *Neoechinorhynchus rutili* (Müller) respectively. Professor Levander very kindly sent me specimens from *Anguilla vulgaris* and I have been able to confirm his determinations with changes to the valid forms of the names mentioned above.

Porta (1905) listed nine species of Acanthocephala from the eel of which two, *Echinorhynchus gadi* and *Acanthocephalus propinquus*, are supposedly of marine origin. However, Porta's records in this work are the result of promiscuous compilation and it is entirely possible that either or both of these species have been misidentified.

The following table gives some of the more important contributions to the knowledge of the acanthocephalan fauna of the European eel.

TABLE I

Acanthocephala Reported from *Anguilla vulgaris*

Species	Porta 1905	Lühe 1911	Levander 1909	Stossich 1885–1898
Neoechinorhynchus rutili	+	+	+	
Acanthocephalus lucii	+	+	+	+
Acanthocephalus anguillae	+	+	+	
Acanthocephalus propinquus	+			+
Echinorhynchus gadi	+			
Echinorhynchus clavula	+	+		
Echinorhynchus lateralis	+			+
Echinorhynchus salmonis		+		
Pomphorhynchus laevis	+	+		
Echinorhynchus miliarius (encysted)	+			+
Corynosoma sp? (encysted)		+		

In the above table are listed eleven species of Acanthocephala from *Anguilla vulgaris*. Two of these are larval forms thus leaving nine species as recorded from the intestine of this host. One of these nine species, *A. propinquus* is restricted to marine fishes while another, *E. gadi*, is considered by European investigators as a typically marine species which is brought into fresh-water only through the agency of migratory fishes. Thus of the species of Acanthocephala infesting *Anguilla vulgaris* two are apparently acquired during the marine phase of its existence and the remaining seven are introduced into the intestine of the eel while it is an inhabitant of fresh-water. From the foregoing it will be seen that the two species of eel differ not only in regard to the species of Acanthocephala harbored but likewise differ in the origin of their infestations. The Acanthocephala of *Anguilla chrysypa* seem to be entirely of fresh-water origin

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while those of *A. vulgaris* comprise a mixture of marine and fresh-water species.

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Explanation of Plate

Each figure is accompanied by a scale which indicates the magnification. The scales accompanying figures 3 and 5 have the value of 1 mm., all others on this plate represent 0.05 mm.

All figures were drawn from stained whole mounts in damar with the aid of a camera lucida.

Fig. 1. Profile view, portion of a single row of hooks from proboscis of *E. thecatus*, showing characteristic cuticular prominence around each hook.

Fig. 2. Proboscis of *N. cylindratus*, surface view, showing characteristic arrangement of hooks.

Fig. 3. Female of *E. coregoni* showing general arrangement of organs. Note especially the lemniscus (*l*) which is shorter than the receptacle of the proboscis (*r*).

Fig. 4. Embryo from body cavity of female of *E. coregoni* from *A. chrysypa*.

Fig. 5. *Tanaorhamphus ambiguus*, type female, showing general arrangement of organs: *b*–brain, *e*–egg mass, *l*–lemniscus, *n*–subcuticular giant nucleus, *r*–receptacle of the proboscis.

Fig. 6. Proboscis of type female of *T. ambiguus*.

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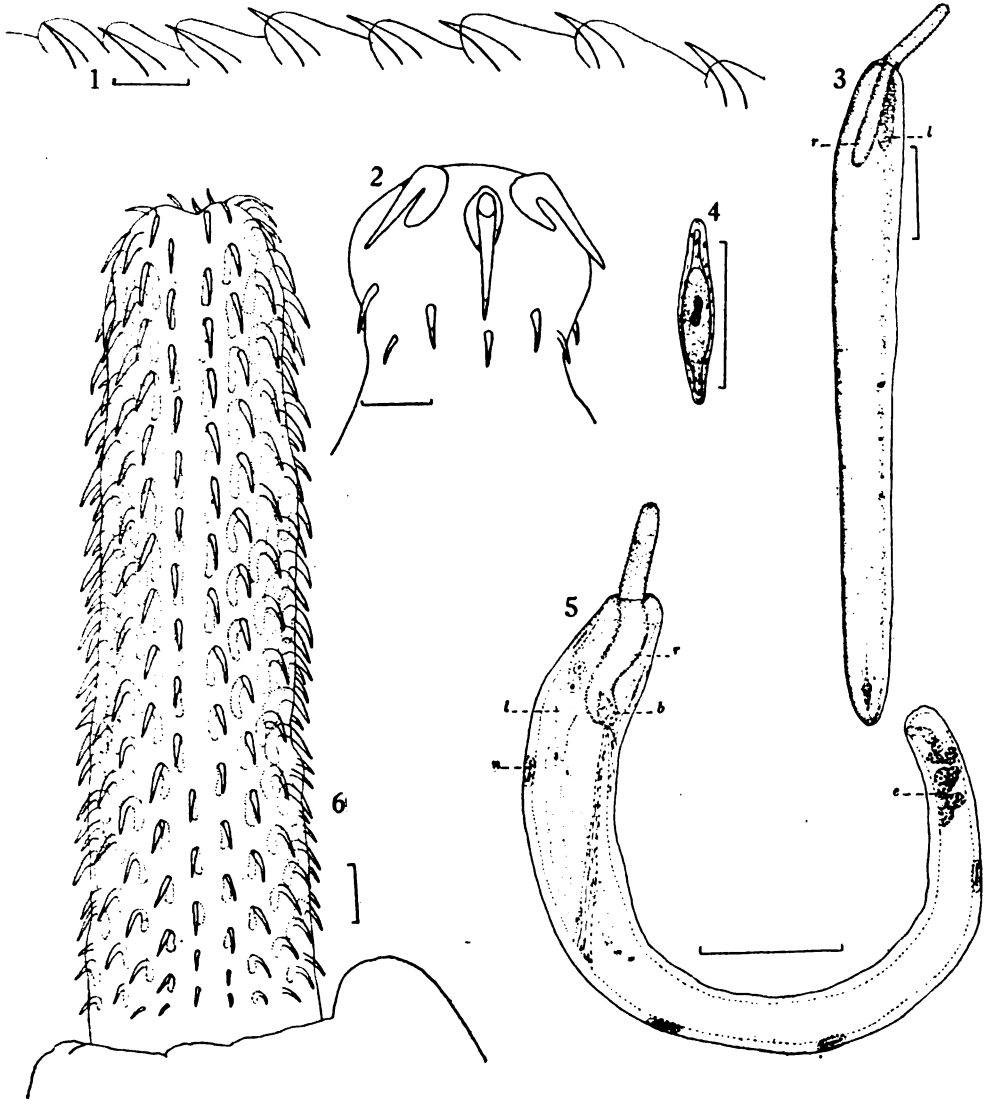

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A BRIEF STUDY OF THE RANGE OF ERROR IN MICRO-ENUMERATION

BY
W. E. ALLEN

Like many other people who have undertaken statistical study of microscopic organisms, I worked for a long time without any very great effort to determine the accuracy of enumeration. This was probably due to five reasons: first, various authorities state that by counting into so many hundreds or thousands, the limits of accuracy are reached, second, because two or three trial counts indicated substantial agreement, third, because the series of counts seemed to follow a normal sequence, fourth, because the insistent demands of routine work made it difficult to undertake a study of this sort, fifth, because it seemed that if great care were observed in handling and counting there was no great probability of improving matters by making such a study.

But it so happened that my co-worker in the Scripps Institution, the late Mr. E. L. Michael, when looking over the manuscript of my paper on the plankton of the San Joaquin River, raised the question as to the accuracy of my counts. We discussed the matter at various times and he always remained sceptical as to my guess that my counts were not in error more than plus or minus ten per cent. So, finally, when I got settled down to my regular program of work on marine phytoplankton, after adopting the measured water method of collecting, it became necessary to get more definite information concerning the accuracy of the counts.

I have made no thorough search of plankton literature for a record of such studies, but I have had access to the most important European and American papers, which I have scanned rather hastily without finding any indication of such a record. Hence, it seemed to me that my experience might be of some value to other workers in this or similar lines. I also thought it might lead some one to make a more thorough study of this interesting problem.

To one who has not given any serious thought to the matter, it may appear that the counting of microscopic organisms is quite similar to the counting of any common objects such as beans or apples. In the case of the plankton organisms, this is not true for several reasons. In the first place, there is usually a certain amount of dirt or débris likely to hide some individuals. Then there is the fact that if one wishes to be sure of getting a required number of the organisms, he must (because he cannot see them) filter a sufficient amount of water to give an actual excess over what he is able to count. He must then (except in the use of one or two highly

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specialized methods) take a fractional part of his catch and estimate the total from the number found in this fractional part. The extraction of the fractional part from the whole and the even spreading of this under the microscope for counting is an important phase in the routine of plankton counting. One can take a pint of beans and after counting the number contained, compute fairly accurately the number in a bushel or a car, but he cannot take the individual organisms one by one from his fractional measure and make such an exact estimate. Furthermore, the microscopic things are necessarily handled in fluid through which they should be nearly uniformly spread for count. If one had to take beans mixed in four or five or one thousand times their volume of water and make the count while they were in the mixture, he might have a little better idea of the difficulty of microscopic counting. Furthermore, there is the matter of eye fatigue and the difficulty of recording the count as it progresses.

A few days after beginning the work of collecting by the measured water method on September 1, 1919, I made a beginning at a study of accuracy of enumeration which I was obliged to discontinue. I did, however, make eight counts of a catch (7728) taken in the forenoon of September 6. The results of these counts are partly summarized in table I.

TABLE I

Eight counts by non standard method, of Catch 7728

No. of Count	1		2		3		4		5		6		7		8		
	Total	Per cent of Deviation	Total	Per cent of Deviation	Total	Per cent of Deviation	Total	Per cent of Deviation	Total	Per cent of Deviation	Total	Per cent of Deviation	Total	Per cent of Deviation	Total	Per cent of Deviation	Average
Diatom cells....	1	91	17	40	8	33	5	41	11	8	3	25	24	100	27	150	12
Dinoflagellate cells.....	350	6	334	1	228	14	292	10	275	17	398	23	406	24	294	11	329
Diatom and Dinoflagellate cells	351	3	351	3	296	13	297	12	286	16	401	15	430	21	321	6	341

The series was not very good because the conditions of counting were not nearly enough alike, first because the first four counts were made on the same day and the other four at intervals of one to four days, second, because the material was kept in the mixing tube throughout the series, merely being shaken up after return of the fractional amount from the slide after each count. Furthermore, the number of diatoms was so small

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as to make that part of the count unreliable and none of the counts were carried quite so far as necessary to give sufficiently dependable results. In spite of these deficiencies the table shows that the series was sufficiently good to be considered statistically significant. Thus it appears that five out of the eight counts of dinoflagellates showed deviations from the mean of less than fifteen per cent and the highest per cent of such deviation was twenty-four. The showing for total numbers of cells is even better, a fact which calls attention to the general probability that a deviation of count in one group may be largely obscured by the count of another group when the two are combined for a total.

Although the table gives great emphasis to the point that the count of such a few individuals as those of the diatoms is valueless as a basis of generalization, it should not be forgotten that such a count may be worth recording because of its positive indication of presence of organisms. Furthermore, the system of random sampling to which we are usually forced, may sometimes lead to just as great differences in estimating the plankton population as is represented here. The significance of both errors becomes rapidly less with increase in numbers of samples.

Constantly harassed by the feeling that I ought to still further improve my basis of judgment as to the values of individual counts, I finally returned to a study of the problem on January 21, 1920, and gave it a large part of my time for the next two months. First I took some care in the selection of a catch for study and finally decided on the one (8102) for 8 P. M. on January 11, 1920, because it showed fairly good representation of both diatoms and dinoflagellates and also because it was relatively free from dirt. First I made ten counts of this catch at intervals of one day or more, the slide being emptied into the mixing tube each time, but the whole being left there instead of being returned to the bottle. The summary of results for this series is shown in table II.

TABLE II

Ten counts of Catch 8102

	Percentage of deviation from the mean									
Number of Count	1	2	3	4	5	6	7	8	9	10
Total Diatom Cells	29	15	10	75	28	5	10	20	2	12
Total Dinoflagellate Cells	4	18	29	3	9	2	33	15	21	18
Above totals combined	23	11	14	58	24	3	11	11	6	13

In this series a few of the more abundant organisms were only counted on one tenth or one twentieth of the slide although most species were

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counted on one fourth. At any rate, there is some probability that the counts of those forms which were most abundant were not carried quite far enough to yield really satisfactory results. Even so, the table shows that in only three counts out of the ten was there more than fifteen per cent deviation from the mean in total numbers of organisms and of diatoms and that there was similar deviation in only four out of the ten totals of dinoflagellates. Stated in another way, the showing is that sixty to seventy per cent of the counts deviated from the mean by not more than plus or minus fifteen per cent.

A momentary inspection of table II shows that the fourth count was the only one in which the deviation exceeded thirty-three per cent and that the enormous deviation in that case was due to some difference in the count of diatoms. Three possible causes of this great deviation

TABLE III
Two counts each of ten successive catches

Catch Number		Regular Count	Recount	Average	Per cent of Deviation
8102	Total Diatoms.....	1112	1814	1463	24
	Total Dinoflagellates...	468	576	522	10
	Combined totals.....	1580	2390	1985	20
8104	Total Diatoms.....	1572	2460	2016	22
	Total Dinoflagellates...	276	440	358	23
	Combined totals.....	1848	2900	2374	22
8105	Total Diatoms.....	2098	2011	2054	2
	Total Dinoflagellates...	358	404	381	6
	Combined totals.....	2456	2415	2435	1
8107	Total Diatoms.....	2898	2132	2515	15
	Total Dinoflagellates...	256	568	412	38
	Combined totals.....	3154	2700	2927	8
8108	Total Diatoms.....	3298	2336	2817	17
	Total Dinoflagellates...	372	394	383	3
	Combined totals.....	3670	2730	3200	15

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TABLE III (*Continued*)
Two counts each of ten successive catches

Catch Number		Regular Count	Recount	Average	Per cent of Deviation
8110	Total Diatoms.....	1602	912	1257	28
	Total Dinoflagellates...	324	426	375	14
	Combined totals.....	1926	1338	1632	12
8111	Total Diatoms.....	682	728	705	3
	Total Dinoflagellates...	164	178	171	4
	Combined totals.....	846	906	876	3
8113	Total Diatoms.....	1516	1386	1451	4
	Total Dinoflagellates...	316	272	294	7
	Combined totals.....	1832	1658	1745	5
8114	Total Diatoms.....	988	1652	1320	25
	Total Dinoflagellates...	424	460	442	4
	Combined totals.....	1412	2112	1762	20
8116	Total Diatoms.....	2760	3348	3054	9
	Total Dinoflagellates...	158	110	134	18
	Combined totals.....	2918	3458	3188	8

require particular mention, first, it is extremely difficult to secure even distribution of the diatoms in the counting cell, second, there was an insufficient count of the more abundant diatoms, third, there may have been a personal error in keeping the tally of the count. My own opinion was that this particular deviation was mainly due to difference in the evenness of spread of the diatoms through the suspending fluid and to insufficient count.

For further test of the matter before making any very definite change in method, I then took ten consecutive catches and made two counts of each. The results are partly shown in table III.

In this table it may be noted that there was only one deviation from the mean of as much as thirty per cent. The fact that this one deviation of thirty-eight per cent was in the count of dinoflagellates might lead

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one to think that dinoflagellates could not be any more readily mixed through the fluid than diatoms. My detailed record of the count shows, however, that this deviation was mainly due to differences in the count of extremely minute forms which I have been including under the name Gymnodinium sp. The difficulty of seeing these forms is quite sufficient to account for this error under the circumstances. It appears, then, from this particular series that the deviation in the count of the fairly visible forms is usually well inside of thirty per cent.

In order to have some basis of judgment as to what increase in accuracy might be expected if counts were made covering the whole slide instead of a fractional part, I then made eight counts of a single catch using four different mounts. For each mount I made one count over the whole slide and one count over one fourth of the slide. The most important results are summarized in table IV.

TABLE IV
Counts of four mounts of Catch 8102

	Percentage of deviation from mean			
Number of mount	1	2	3	4
	Percentage of deviation in full slide counts			
Diatom colonies	18	5	3	25
Diatom cells	11	8	3	0
Dinoflagellate cells	5	3	3	5
Total cells	10	7	2	2
	Percentage of deviation in fourth of slide counts			
Diatom colonies	47	30	12	16
Diatom cells	30	53	25	25
Dinoflagellate cells	8	12	12	8
Total cells	25	35	20	33

While the four counts of each kind are not enough for definite conclusions, they are quite suggestive. It was not practicable to carry the series further because of the great amount of time required. As it is there is strong indication that under usual conditions the count covering the full slide is much more likely to approach the mean than is the count made over some part only.

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After giving the matter a good deal of thought, I came to the conclusion that by standardizing mixing processes, much could be done toward reducing the errors of the fractional counts. I, therefore, adopted the practice of shaking the storage bottle for one minute before pouring the contents into a mixing tube, and of reversing twenty times each mixing tube used. All other manipulations had already been made as nearly uniform as possible.

I then selected for study catch number 8104 of 8 A. M., January 12, because of its close resemblance to 8102 which had become somewhat unreliable from repeated handling. Twenty counts were made of samples from this catch. At least twenty-four hours intervened between each two counts and the total catch was returned to the storage bottle after each count so that the sampling might be done in approximately the same way each time. With the first ten counts a test was made of the method of selecting fractional areas in the cell. In one case the areas were selected at intervals around the margin and in the other a median zone lengthwise of the cell and covering one fourth of its area was selected. The second ten counts were made by the median zone method but record was kept of the numbers at areas of one fifth as well as of one fourth of the slide. The results are summarized in tables V and VI.

Table V shows the percentage of deviation from the mean by marginal (twentieth to fourth of slide) and median (fourth of slide) counts in the first ten counts, calculated from the mean for this ten, by fifth and fourth of slide counts in the second ten calculated from the mean for that ten and by fourth of slide counts in the twenty counts calculated from the mean for the whole twenty. Without attempting extended analysis of the tables, I may call attention to the fact that the deviations shown by ten counts do not indicate very much difference in most cases between the marginal count (which varied from 1/20 to ¼ of the slide) and the fourth of slide count, nor between the fifth of slide and fourth of slide counts, but that there is a much greater range of deviation in the marginal counts. I also note the fact that there is a better approximation to the mean in the fourth of slide counts in the case of Gonyaulax polyedra, which is a dinoflagellate of sub-globular form. Such a difference in count of this organism might be expected because its shape would favor fairly even distribution in mixing and handling while most other organisms are sufficiently irregular in form to lead one to expect them to be more erratic in any distribution undertaken by shaking or stirring of the surrounding fluid. In the twenty count series it may be noted that the difference between Gonyaulax and total dinoflagellates tends to disappear but that the difference between both and diatoms is accentuated. The close resemblance of Gonyaulax to total dinoflagellates is attributable largely to the fact that Gonyaulax contributed about two thirds of the total.

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The increased difference in range of deviation between Gonyaulax and the total diatoms is explicable on the basis of what has just been said as to differences in distribution due to form.

TABLE V—Catch 8104—Percentages of deviation from the mean

	Diatom colonies			Diatom cells			Dinoflagellate cells			Total cells			Gonyaulax polyedra		
		Median ¼ No. of counts			Median ¼ No. of counts			Median ¼ No. of counts			Median ¼ No. of counts			Median ¼ No. of counts	
	Marginal count	10	20	Marginal count	10	20	Marginal count	10	20	Marginal count	10	20	Marginal count	10	20
1	6	9	19	1	8	16	6	6	10	0	8	16	29	7	2
2	12	12	22	17	10	18	21	3	1	12	8	16	21	3	1
3	30	13	1	15	5	4	15	10	6	49	6	3	11	1	5
4	7	0	11	9	1	10	12	7	11	10	2	11	23	14	18
5	6	1	11	12	8	1	34	12	15	16	6	3	27	3	7
6	4	3	8	0	11	1	23	3	7	3	10	0	5	16	12
7	7	12	21	0	16	24	0	8	3	0	14	21	13	7	11
8	24	14	23	37	29	36	0	10	13	33	27	33	5	12	16
9	7	5	16	11	15	23	4	7	2	10	12	20	5	5	0
10	31	38	23	35	56	42	15	9	5	28	50	37	19	5	0
	Median 1/5 of slide	10		Median 1/5 of slide	10		Median 1/5 of slide	10		Median 1/5 of slide	10		Median 1/5 of slide	10	
11	0	1	10	14	15	26	4	5	10	13	14	24	13	12	17
12	2	1	10	4	1	8	6	0	3	4	1	6	18	7	3
13	13	12	3	6	7	0	10	2	6	4	6	1	10	2	6
14	7	5	17	9	1	10	5	0	3	7	1	9	1	2	2
15	8	4	15	3	2	7	5	6	2	3	2	6	4	4	0
16	11	10	21	12	5	14	12	13	9	10	3	12	1	7	3
17	8	13	4	1	6	3	5	3	1	2	5	2	16	13	9
18	5	3	8	0	4	4	4	0	4	0	4	4	8	2	6
19	13	6	18	19	8	17	4	10	14	18	8	17	1	7	12
20	5	5	17	4	8	0	10	6	9	2	6	2	13	10	13

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Table VI covers some of the same ground as table V but in a different way. In this table enumeration totals are shown instead of percentages, with the addition of a list of numbers of Gonyaulax polyedra in each of the twenty counts and a list of numbers of both cells and colonies of Nitzschia seriata in each of the twenty counts. It also includes a statistical summary which the late Mr. E. L. Michael very kindly prepared for me. The series is too short for statistical treatment but the summary has some interest in a suggestive way.

This summary indicates that the extreme deviation is not only more than twice as great in the case of diatom cells as it is in the case of Gonyaulax and total dinoflagellates but that the same thing is true of both cells and colonies of Nitzschia seriata, the most abundant diatom in the catch. Nitzschia seriata is a slender spindle-shaped diatom occurring very largely in colonies of two to six individuals. Its form would lead me to expect it to be quite erratic in distribution by any possible method of mixing. This is also to be expected of the other numerous diatoms, which belong mainly to the Chaetoceras group. It is also interesting to note that the wide range of deviation in diatoms is due to the tenth and eleventh counts and that in count ten the numbers of both dinoflagellates and Gonyaulax are very close to the mean, though Gonyaulax approaches the extreme deviation in the eleventh count.

This last point is important because of its indication that the error lies in the mixing and distributing of the organisms rather than in the method of counting. The normal count of the less erratic Gonyaulax indicates that there was no serious mistake in counting, computing or recording, while the known erratic distribution of the diatoms does indicate considerable variability in results of mixing. In spite of the large extreme deviation due to diatoms, the mean variability for total cells is only 12.2%, a fact which gives ground for thinking that totals of most counts are within a range of error of less than ten per cent.

A point which can be verified by the reader in table VI, but not in the others (though true of all), is that the deviations are fairly evenly distributed on both sides of the mean. This is an indication in this type of study that the fluctuations are normal and that they appear approximately according to expectation.

Although this study as a whole is distinctly brief and fragmentary it seems to give a good practical basis for the following provisional conclusions: First, that by very great care the extreme deviation (in total numbers of diatoms and dinoflagellates) could probably be kept within twenty-five per cent; second, that the mean deviation can be easily kept within ten per cent; third, that diatoms are more variable in the counts than dinoflagellates; fourth, that the causes of variability are to be found in the processes of mixing, sampling and spreading on the slide, rather

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TABLE VI

Catch 8104

Enumeration totals, deviations, etc.

Count	Total Diatom Colonies	Total Diatom Cells	Total Dinoflagellate cells	Total Cells	Gonyaulax polyedra	Nitzschia seriata
1st	696	2152	304	2456	220	Col. 280 Cells 660
2nd	676	2100	332	2432	212	188 372
3rd	872	2464	356	2820	204	292 560
4th	768	2300	300	2600	176	232 436
5th	764	2532	284	2816	200	252 604
6th	792	2604	312	2916	240	220 552
7th	680	1948	348	2296	192	160 372
8th	664	1652	292	1944	180	240 536
9th	728	1984	344	2328	216	264 424
10th	1060	3644	352	3996	216	308 648
11th	948	3232	368	3600	252	304 796
12th	952	2770	348	3118	208	264 532
13th	840	2596	356	2952	228	232 508
14th	1008	2828	348	3176	220	224 384

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TABLE VI (*Continued*)
Catch 8104
Enumeration totals, deviations, etc.

Count	Total Diatom Colonies	Total Diatom Cells	Total Dinoflagellate cells	Total Cells	Gonyaulax polyedra	Nitzschia seriata
15th	992	2760	328	3088	216	336 608
16th	1048	2940	304	3244	208	316 632
17th	832	2640	340	2980	196	252 564
18th	932	2676	348	3024	228	284 556
19th	1016	3016	384	3400	240	304 738
20th	1008	2592	368	2960	244	304 616
Extreme deviation	246=30.2%	1059=41%	52=15.5%	1089=37.4%	39=18.2%	103=39.1% 242=43.7%
Average	814	2585	336	2907	215	263 554
Standard deviation	142	463	27	410	20	44 112
Average deviation	124=15.2%	358=13.9%	23=6.9%	354=12.2%	16=7.5%	37=14% 87=15.7%

than in the counting; and fifth, that the range of error in counting is at worst far less for microplankton material than is the range of error in locating, catching and preserving material.

It seems fair to regard these results as suggestive for microscopic material in general, e. g., enumeration of blood corpuscles might be expected to show a range of error somewhat similar to that of Gonyaulax and direct enumeration of bacilli to give results resembling those from diatoms.

As regards my own use of the study, I may say that it has led me to decide on the mixing procedure already mentioned, and in counting to

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carry all enumerations to fifty individuals (or fifty colonies) or to a very close approach to fifty at a convenient computing point, except that all enumerations are stopped when one eighth of the slide has been covered.

I may say frankly that for a single count or for a very short series of counts, this number limit and area limit are too small. But in handling large numbers of catches in large series and working through long periods of time, one must give close attention to the law of diminishing returns. Would the counting of a larger number of abundant forms or the counting of all over a larger area give enough greater approach to accuracy to compensate for the greater effort and use of time? It has not seemed to me that it would for present purposes. With the lens combination on a monocular microscope which was used in making this study, it was convenient to work over the area of one fourth of the slide. Later when using a different lens combination on a binocular microscope, it was found that an area of one eighth of the slide was more convenient. In fact some counts are so fatiguing and so time consuming at one fourth slide as to be impracticable in a long series. With my present standardized procedure I should expect the one eighth slide counts to show about the same range of error as indicated for the one fifth slide counts in table V. I have not yet had time to verify this assumption. At worst the range of error in careful work will certainly not be as great as that due to other factors as far as microplankton is concerned.

Finally, I may say that although the results which I have obtained are inadequate for definite conclusions, they do indicate that with standardized procedure the microscope phase of plankton study is much more nearly accurate than some of the other phases.

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

REMARKS ON THE LIFE-HISTORY AND THE SCALE CHARACTERS OF AMERICAN MULLETS

BY

CARL L. HUBBS

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Mr. Arthur Paul Jacot, in the July issue of these TRANSACTIONS for 1920 (pp. 199–229), has presented the results of his investigations on scales of two American mullets: *Mugil cephalus* and *M. curema*. He has discovered a number of facts bearing significantly upon problems in several of the zoological sub-sciences. These facts, and, more particularly the author's interpretations of them, are discussed in this brief note.

Mr. Jacot's discovery that the scales of these species of *Mugil* are ctenoid proves an unexpected confirmation of the view recently held by Jordan and Hubbs[1] that the group Percesoces, comprising the Mugilidae and related families, is derived from the typical Acanthopterygii (which is characterized in part by ctenoid scales), and hence is not transitional between the cycloid-scaled malacopterygian fishes and the more specialized spiny-rayed types. The wide differences found in the character of the ctenii on the scales of the species of Mugil studied are also of considerable taxonomic interest.

The detailed account given of the development of scale structure, and the final proof of the transformation of the first soft-ray of the anal fin of the juvenile or Querimana stage into the third anal spine of the adult *Mugil*, are valuable contributions from the standpoint of the comparative anatomy of these structures. This juvenile metamorphosis of *Mugil* has long been in need of the detailed study which Mr. Jacot has accorded it.

The sharply defined mark on the scales of *Mugil cephalus*, which the author appears to have interpreted as a metamorphic annulus, is obviously the first winter annulus; apparently intensified, it is true, by the fact the adult characters appear first in the spring, synchronously with the resumption of the growth of the scale and of the fish, following the cessation of growth significantly demonstrated to occur during the winter. The portion of the scale within this first winter annulus therefore corresponds with the

[1] A monographic review of the family of Atherinidae or Silversides (Stanford Univ. Publ.), 1919.

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brief period of initial growth[2] between hatching and winter. The similarity existing between the first annulus developed on the scales of *Mugil curema* to that of *M. cephalus* indicates that this species likewise breeds in the fall, rather than during the summer as Jacot supposed.

This altered conception of the nature of the first annulus involves a different interpretation of the age at maturity of *Mugil cephalus:* the first spawning fish appear to be just two years old (rather than in their second year); similarly, the oldest individual examined was six years old.

The second and succeeding line-like annuli developed on the scales of these species of *Mugil* being typical of the winter marks developed on the scales of marine fishes of temperate waters, and of the coregonine fishes of the Great Lakes, it is, to say the least, unnecessary to follow Jacot in interpreting these marks as migratory rather than as winter annuli. The fact that an annulus was not evident near the margin of scales of mullets taken at Beaufort in early spring indicates merely that the spring growth of these fishes had not yet commenced, and not that these fishes were exceptional non-migratory individuals. Indeed, it is not at all certain that the mullets actually do migrate southward during the winter, for a growing body of evidence is indicating that in this season many shore-fishes of the Temperate Zone merely retreat into deeper water and become less active, and hence appear absent because not caught.

These altered interpretations bring Mr. Jacot's facts into much better agreement with the results of studies made on the life-history of other fishes, and in the opinion of the writer, enhance the value of his contributions.

Mr. Jacot has introduced some new terms, none of which will probably be adopted. Of these "adulting (changing to the adult condition)" and "circulation" (referring to the course of the circuli on the scales), require no further comment. The term "linea (from the Latin *linea*, ae, f.; using the term in its more figurative expression)," is unnecessarily substituted for *annulus* or "winter band"; a similar statement might be applied to "ctenobasii."

[2] It is probable that the juvenile mullets pass during this period through a pelagic stage, for which the Querimana characters are well adapted. *Labidesthes sicculus*, a fresh-water fish related to *Mugil*, passes through such an initial pelagic stage.

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SPRING MIGRATION IN THE CRAYFISH, *CAMBARUS ARGILLICOLA* FAXON

BY

HAROLD CUMMINS
Tulane University

Incidental to a study of the migration of frogs into their breeding ponds, carried out near Ann Arbor, Michigan, in the spring of 1914, some interesting observations were made upon migratory activities of this burrowing crayfish. So little is published regarding the habits of crayfishes that even these few notes seem to be worthy of publication.

The location and character of the pond and the method of obtaining migration data by trapping are described in another paper.[1] Briefly it may be said that the pond is at the edge of a cultivated field, bounded partially by a wood which adjoins the field. About this pond a trap was constructed, extending approximately two-thirds of its circumference. The trap consisted of a cloth fence, provided with leaders of similar construction extending radially outward from the main fence. At the junction of each leader and the main fence a pail half-filled with water was sunk in the earth with its top at ground level. Crayfishes migrating toward the pond came in contact with this cloth barrier, and as they edged along it in an attempt to enter the pond were entrapped in the pails. Since the pond was not completely enclosed by the trap the number of crayfishes taken does not necessarily represent the total number of migrants; some may have gained entrance where the fence was incomplete.

White's Wood, at the edge of which the pond is located, fulfills the habitat requirements of this species, and so far as the writer's collections indicate, *C. argillicola* is the only crayfish that occurs there. In addition to the observation pond there are four small ponds in the wood, two of which like the observation pond are usually not dried in the summer. The remaining two always dry during the summer. All of them are frequented by crayfishes. The burrows, usually with chimneys, form a characteristic feature of the habitat; sometimes they are found at some distance from the ponds, but usually near them, and when the ponds dry numbers of chimneys are thrown up on the exposed mud.

After their winter torpidity was dissipated by warm weather, crayfishes reappeared in the pond, not only from the bottom of the pond itself but also from outside sources. The first individuals to appear were those which had spent the winter in burrows in the pond bottom. On March 23 the burrows were first opened to the surface, numbers of them being

[1] Cummins, Harold, The rôle of voice and coloration in spring migration and sex recognition in frogs. Jour. Exp. Zool., v. 30, no. 3, April 1920.

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observed for the first time on that date. Each opening was circular, averaging 1.5 cm. in diameter. Bordering the opening of each burrow was an approximately circular area, averaging 9 cm. in diameter, of light-colored sand, apparently brought up from a lower level in the process of opening the burrow. The sand was not thrown into a high convex mass, but rather was so small in quantity as to be not appreciably elevated from the level of the pond bottom. Unfortunately no attempt was made to collect crayfishes from the pond before March 23, therefore it is impossible to state whether or not all individuals spent the winter in burrows. Data on the reappearance of crayfishes which were outside of the pond during the winter, presumably in their burrows, were obtained from the trap. The results of the trap are presented in the accompanying chart.

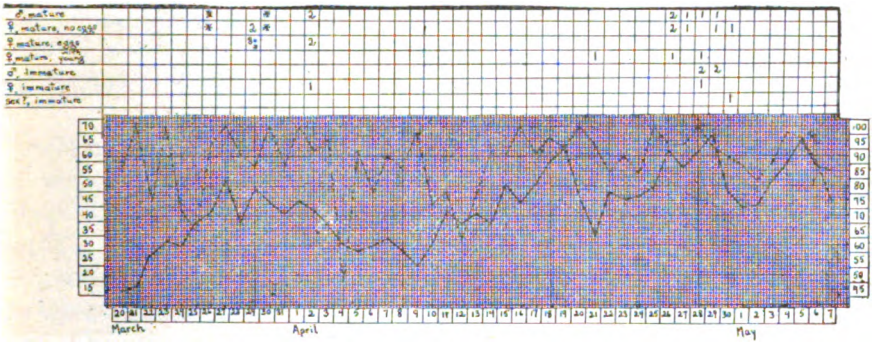

Chart showing the trap catch from March 26 to April 30, inclusive, and temperature and humidity records from March 20 to May 7, inclusive. Temperatures in the Fahrenheit scale are indicated at the left, and the average temperature for the 24 hours ending 7 A. M. of each day is plotted in a continuous line on the graph. Degrees of relative humidity are indicated at the right, and the average for the 24 hours ending 7 A. M. of each day is plotted in a broken line on the graph. In several instances the number of trapped crayfishes was recorded in the field notes as "several"; such records are here shown by the asterisk (*).

It is evident from the chart that migration occurs at irregular intervals. There is a broken migration wave from March 26 to April 2, inclusive, and an unbroken wave from April 26 to 30, inclusive, while in the period from April 3 through April 25 but one crayfish was trapped. The migration waves occurred during periods of relatively high temperature and humidity. The lowest temperature with which a migration is coincident is 33.6 degrees (April 21), but with only one individual. A temperature more favorable to migration, if judgment can be based on the trap catch, is 42 degrees or over. All the catches are coincident with high humidities, the lowest being 87 (March 29), the others ranging between 90 and 100.

With the exception of the female carrying young, captured on April 21, all the collections were made in the morning, representing crayfishes

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migrating during the preceding night. The single exception is noteworthy in demonstrating that spring migratory activity may occur in the daytime. This migrant was not actually trapped, but was noted at 10 A. M. walking in the grass near the edge of the pond. In view of the remaining migrations having been nocturnal, probably this record represents a crayfish which migrated during the preceding night, and, coming in contact with the fence, walked away instead of alongside as did others.

The small number of immature individuals is suggestive. One trapped April 30 was only an inch in length, and therefore unquestionably not sexually mature. The remaining six were about two-thirds the size of average adults. Whether there is some stimulus controlling the migration of adults, which is usually lacking in the young, or whether the young will migrate later are questions which cannot be answered with the data at hand. The facts of an early beginning of migration among adults and the retardation of migration of all but one of the immature crayfishes leads to the inference that migratory impulses occur both in young and adults, but begin to function earlier in the season in the latter.

A total of ten plus "several" females with eggs were captured on March 29 and April 2, and none appeared thereafter. During the first migration wave several females without eggs (two plus two lots of "several" each) were trapped, and in the second wave five without eggs appeared. Three females carrying young appeared in the second wave. The bearing of these data upon the time and place of egg-laying and hatching is important, but difficult of interpretation. If those females bearing eggs and young furnish a standard of comparison, we must assume either that there is a prolonged period for the egg-laying and hatching of the last five females or that their eggs already had hatched. The same question does not arise in connection with the females without eggs which were captured early. It seems that a migratory movement of adult females in the spring would prove advantageous to the young, for they would hatch in water which presumably provides a more favorable environment for them than the burrows.

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PREPARING COLLECTIONS OF THE MOLLUSCA FOR EXHIBITION AND STUDY[1]

BY

FRANK COLLINS BAKER

Curator, Museum of Natural History, University of Illinois

The Mollusca form a large group of the Animal Kingdom and members of this phylum are used for economic or biologic study by many biologists, zoologists, geologists, ecologists, and others interested in the study of animal life. Collections are also made for their beauty or interest by amateur students. Whatever the cause of interest it is important that the collections made should be properly prepared and preserved for future consultation. The good appearance and permanence of a collection of mollusks depend very largely upon the care taken in cleaning and preparing the individual specimens. The modus operandi varies with the size and the kind of mollusk.

CLEANING THE SPECIMENS

Mussels or River Clams. The river mussels, when only the shells are to be preserved, should be placed in boiling water which will cause the valves to open slightly. The adductor muscles may be cut with a thin-bladed knife and the animal matter removed. Care should be taken to remove all of the animal matter from the region of the muscles where it is strongly fastened. During this process the collector must avoid breaking or injuring the edge of the shell where the substance is very thin, the new shelly matter as well as the epidermis or periostracum being newly formed at this part of the shell. This is especially true of the thin-shelled mussels like *Anodonta*. After removing the animal parts the shells should be washed carefully to remove the mucus and any parts of the animal remaining. Care must be exercised to avoid breaking the ligament which holds the two valves of the shell together. When thoroughly cleaned the two valves may be tied together with *white* string (*never* use colored string for it will mark the shells) and the shells laid on boards or other objects to dry in a warm place. Never allow the sun to shine on specimens of this kind for they will then dry too quickly and the epidermis will peel off. A few shells of each lot should be broken apart so that the interior, especially the hinge structure, may be studied.

Many shells will be marred by incrustations of lime or other matter. This may be removed with muriatic or oxalic acid, which may be applied with a small camel's hair brush. As these acids, especially muriatic acid, readily attack that part of the shell not protected by the horny epidermis,

[1] Contribution from the Museum of Natural History, University of Illinois, No. 17.

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the specimens should be washed carefully and quickly after using the acid. Many shells may need to be scrubbed with a small scrubbing brush or a nail brush to remove the extraneous matter. In some cases, however, it may be desirable to preserve the shells in their natural state, with all the incrustations and other foreign matter attached, to indicate the character of the water or bottom in which the animals lived. This may be necessary in some ecological studies. After the shells are thoroughly dry the strings may be removed and the surface of the shells rubbed with vaseline. This will usually prevent the epidermis from peeling or cracking and will give the shell the appearance it had when living in the water. Great care should be used to see that all of the surplus vaseline is removed or the surface will become sticky and unsightly. A soft rag may be used to rub the shells perfectly dry and clean.

Finger-nail Clams-Sphaeriidae. The smaller bivalves—*Sphaerium, Musculium, Pisidium*—are usually too small for the animal to be removed from the shell and they may be killed in 70 per cent alcohol from which they may be removed and dried in a few days. In the case of the larger *Sphaerium* the animal may be removed, after having been killed by boiling or by preservation in alcohol for a few days. As the valves of the shell are liable to open after being cleaned, and as they are usually too small to be tied together, they may be wrapped tightly in a plain piece of tissue paper until dry, when the paper may be removed. No oil or other preservative should be used for these shells.

Fresh Water Univalves or Snails. The larger fresh water snails may be killed by boiling or by preservation for a few days in 70 or 80 per cent alcohol. The animals are then easily extracted with a dissecting needle. A needle with a curved or twisted point is more effective in removing the animal from the inner whorls than one with a straight point. In the large *Lymnaea, Planorbis,* and *Physa,* the animals are easily removable, but in the *Pleurocera, Campeloma,* and related genera, the animals must be removed with great care as the upper part of the animal, containing the liver and part of the sexual organs, is liable to break off and remain in the shell. When removing these animals, get a firm hold of the body with the dissecting needle and then by a slow, careful, twisting motion remove the animal. All animal matter should be removed from the large shells.

If there are incrustations or other foreign material on the surface of the shells this may be taken off with a brush, scraped off with a knife, or removed with the acids mentioned for mussel shells, oxalic acid being the best. The acids must be used with care that the fine texture of the shells may not be injured. In those species having an operculum, like *Campeloma* and *Pleurocera,* the opercula of a few individuals of each lot should be removed from the foot of the snail, dried, and placed inside the aperture of the shell, which may then be closed with a piece of fine cotton. It is

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not a good idea to glue the operculum to the cotton because the inner side which bears the muscle scars for its attachment to the operculigerous lobe of the animal may be needed for study. All shells should be thoroughly dried before placing them in the cabinet and before placing the operculum in the aperture. It is well in the larger *Campeloma* and *Vivipara*, to wipe the surface gently with the rag used for vaselining the mussel shells, using the same care as recommended for that group in this particular. The smaller snails, *Amnicola*, *Valvata*, small Lymnaeas (*Galba*), *Ancylus*, etc., may be killed in 70 per cent alcohol, from which they may be removed in a few days and dried. The little fresh water limpets (*Ancylus*) should have the animal carefully removed with the point of the dissecting needle. As these small limpets are usually coated with foreign matter they may be effectively cleaned by being allowed to float, upside down, on the surface of a small quantity of oxalic acid, after which they may be washed and carefully wiped with a camel's hair brush. The shell is thus easily cleaned if held, aperture downward, on the tip of the index finger.

Land Shells. The larger land snails or Helices should be placed in warm water which should be quickly brought to the boiling point to kill the animals. It is of importance to be certain that the water is boiling for hot water will not kill the animal at once and it will then be difficult to remove from the shell. Land shells cannot be left too long in the boiling water because the fore part of the body is liable to break away from the part containing the liver, which will then remain in the upper whorls of the shell and be very difficult to remove. If not killed quickly by boiling, the columella muscle will not be loosened from the pillar lip and the animal cannot be pulled out without breaking in pieces. The larger species must be boiled for fully a minute but the smaller species, the size of *Polygyra hirsuta*, will be ready to have the animal removed in 10 or 15 seconds. To prevent loss in a large tin or pot it is well to place the snails to be boiled in a wire dipper which may be obtained in any 10 cent store.

To insure successful extraction of the animals it is necessary to use great care and plenty of time. The same curved dissecting needle mentioned previously is well suited for removing the animals of land snails, and the same twisting motion is necessary as described under fresh water snails. If the animal breaks during the operation, leaving a portion in the upper whorls of the shell, the remaining part may be removed with jets of water from a small syringe, preferably a fine-pointed dental syringe. It may be well sometimes to place the shell in alcohol for a day or two in order that the part of the animal left in the shell may be loosened, after which the syringe will usually remove the matter. Sometimes a vigorous shaking, or, with the hand holding the shell, striking the other hand or the thigh, will aid in loosening the refractory matter. Much

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patience and some ingenuity is necessary in removing the animals from their shells in which the aperture is restricted or contracted by teeth or folds, and in these cases the fine syringe will be found useful to start the body from the shell. All shells should be washed out inside with the syringe and scrubbed on the outside with a tooth brush, or other small brush, to remove all traces of mucus, dirt, or other foreign matter. A gentle flow of water from a tap or faucet is very effectual in removing mucus and dirt from the interior of large shells. If the mucus is unusually adhesive, as is sometimes the case, it may be necessary to use a small piece of sponge or cotton attached to the curved dissecting needle, or held with a pair of curved forceps, to remove the unsightly material. Land shells do not require vaseline for the preservation of the epidermis as suggested for fresh water mussels and large water snails. When perfectly clean the shells may be laid on boards or other objects and laid in a convenient place to dry. Never allow shells to dry in the sun for they will crack and be spoiled for cabinet purposes. Too strong emphasis cannot be laid on the injunction to remove *all* animal matter from the larger land shells, which have a peculiarly offensive odor all their own if placed in a cabinet only partly cleaned.

The small land snails, especially the members of the Pupillidae and those snails having teeth or folds in the aperture, cannot well have the animals removed. If these are kept for a few days in a dry place the animal will retract well within the shell and they may then be placed in 30 or 40 per cent alcohol for twenty-four hours, after which they may be dried and no offensive odor will be retained. Vermin will not usually attack a shell that is thus well soaked in alcohol. When dirt of any kind remains attached to these small shells they may be effectually cleaned by being put in a bottle with fine, clean sand, and a vigorous shaking will remove the dirt. This process should not be used for fragile shells. It is especially effective with the Pupillidae.

Marine Shells. The directions given above for land and fresh water shells apply equally well for marine mollusks. The snails from the sea, however, are more difficult to prepare because of the more powerful columellar muscle by which the animal is attached to the shell. For the larger species of sea snails the curved dissecting needle will hardly be adequate to extract the animal. For this purpose nothing is better than a stout fish hook which has been heated and then bent in the form of a partial spiral. Plunging in cold water after shaping will return the temper of the steel sufficiently for the purpose for which it is made. The shank may be firmly fastened in a wooden handle made in convenient shape to fit the hand, and the result is a very useful implement. In extracting the larger animals from their shells, it is important that the hook be deeply and firmly buried in the large, tough muscle attached to the columella

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pillar or axis of the shell. A strong, steady pull will usually bring the animal.

Bivalve shells, clams, may be treated in a similar manner to Unionidae mentioned on a previous page. Boring clams, like *Pholas*, *Teredo*, and others, will require special attention to preserve the extra pieces of shelly matter connected with shell. Small clams may be treated in the same manner as mentioned under finger-nail shells. The same may be said of the small snails which should be treated as the small fresh water or land snails. Marine shells may be killed in boiling water or by preservation in alcohol. As in the case of land and fresh water mollusks, formalin is not a good preservative on account of its action on the shells.

Many marine snails are encrusted with limy matter, the tubes of worms, the hard shelly bases of corallines, and the dried remains of sponges. These may be removed with an old file the end of which has been ground to a point. Little chisels and punches like engraver's tools are also excellent for this purpose. With care and experience the collector will be able to scale off the greater part of this extraneous matter without harming the shell beneath. The judicious use of muriatic acid will also help in the final cleaning process, but this reagent must be used with great discretion in order not to mar the surface of the shell.

Preparation for Anatomical Study

It is frequently desirable that some of the material collected should be preserved for the study of the animal. Fresh water pulmonates, such as *Lymnaea*, *Planorbis*, *Physa*, may be placed directly in 30 per cent alcohol, where they may remain for twenty-four hours. They should then be placed in 50 per cent alcohol for another twenty-four hours, and finally preserved in 75 or 80 per cent alcohol. The fresh water operculate snails may be preserved in the same manner, as may also most of the marine snail shells.

Land shells, however, must be killed in osmic acid or by drowning, the latter being the best, causing the animal to die in a fully expanded condition. For drowning, the writer has obtained the best results by placing the snails in a large, wide-mouthed bottle, filling the bottle level full with water and placing a heavy piece of glass over the water to exclude all air bubbles. In twelve to twenty-four hours the animals will be fully expanded and quite dead and may then be removed to 30, 50, and 80 per cent alcohol as recommended above for fresh water snails. Care must be exercised that the snails are not taken from the drowning water too soon, for in this case they will contract badly when placed in alcohol.

Final preservation may be made in a 2 per cent solution of formaldehyde, but alcohol is better for the flexibility of the animal, which has a

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tendency to harden and become brittle in formaldehyde. Even in a weak solution of formaldehyde the shells gradually soften and easily break when handled. A recent examination of some molluscan material in a research collection of a well-known laboratory was found to be time wasted because the material had been preserved in formaldehyde, and the shells had softened and curled up, almost entirely losing their original character. Valuable material upon which scientific conclusions are based is thus liable to be ruined for future study and examination.

Slugs (*Limax*, etc.) and snails with small or very thin shells may be preserved as mentioned for the animals of land shells. The eggs of all mollusks, fresh water as well as land, should be preserved in alcohol, after passing through the different grades of the preservative. Some eggs, as those of *Pyramidula* and *Polygyra*, have a more or less hard shell and may be dried and preserved in bottles. In the case of large eggs of the Bulimi and other large land shells, they must be treated in the same manner as birds' eggs and the contents removed by means of an egg blow pipe. They may then be dried and placed in the collection.

For bringing out details of the surface structure of snails a 1 per cent solution of chromic acid has been found to be a good reagent. Müller's fluid is also an excellent fixing reagent. These reagents, however, harden the body to such an extent that it is often difficult to make gross anatomical examinations and the alcohol method described above is the best for all purposes. When using the fixing reagents mentioned it is highly important that the animals be washed thoroughly in running water before being transferred to the different grades of alcohol. Twelve to forty-eight hours will be necessary for this purpose, depending upon the size of the specimen treated. No specimens should be placed in strong alcohol at once as this reagent extracts the water so rapidly that the internal organs are shrunken and distorted. For sectioning and some histological purposes the hardening methods mentioned are excellent.

Preservation for Study or Exhibition

The method of preserving and arranging a collection of mollusks will depend wholly upon the purpose for which it was made. All collections may be roughly divided into two types, those for display and those for study. Each of these types requires a different treatment.

Collections for Display. Collections of this kind will probably be confined almost exclusively to museums of one kind or another. An exhibition collection of the Mollusca, even in a public museum, should be more or less synoptic in character, and arranged to show the principal features of classification, as well as facts relative to different kinds of habitats—ponds, rivers, swamps, shallow water, deep water, rocky shores,

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sandy shores, forests, plains, and valleys—in short, the ecology of this type of animals. The geographic distribution—Arctic, temperate, tropic, island, continental, etc.—should be indicated by charts; the variation of individuals and the economic use made of certain species should also be clearly indicated. For some of this display, models may be used to illustrate ecology, geographic variation, and methods of life. Features of this kind add much to the value of a collection and are always interesting to those persons visiting a museum that are not particularly interested in the general subject of mollusks. Such economic displays as pearl buttons and the clams from which they are made, both fresh water and marine, shell money as used by the native tribes of this and other countries, mollusks used for food, injurious snails, pearls, and other topics of like nature, are very interesting, useful, and highly educational.

For exhibiting mollusks a strong, durable, attractive tablet is essential. Such an one can be made of heavy binder's board (no. 20) cut into convenient sizes and covered with such material as will give the best effect to the collection. Many shells will look well on a black background and these may be mounted on tablets that have been covered with a dull black paper. Dark shells look better on a light background, and for these the writer has used an ivory-colored cardboard known as Royal Worcester Bristol Board, a material that withstands the fading power of light better than any other paper used. For these light backgrounds the cardboard is cut just a trifle smaller than the tablet, the edges of which have previously been passépartouted with a dead black paper used for binding together lantern slides, and the light cardboard is glued to the tablet (glue being used only about the margin of the card), leaving a border of black. This method produces a handsome tablet that is both durable and attractive. When the label is attached (which should be made of the same cardboard used for the center of the tablet) the whole has a pleasing appearance. The sizes of these tablets, as used by the writer in his museum work, and found to be the most useful, may be 3 x 2, 3 x 3, 3 x 4, 3 x 6, 3 x 9, 6 x 6, 9 x 9, and 12 x 12 inches. All of these are multiples of the small unit, 3 x 2 inches.

To make an exhibit collection of the greatest value from a teaching standpoint, many drawings of structure and development, maps of distribution, and labels describing the function of organs, as well as notes of interest concerning the animals or shells, should be freely used. A famous museum man, Dr. G. Brown Goode, once said that a museum was a collection of labels illustrated by specimens, and while this axiom is pretty strong and the matter may be somewhat overdone, the fact nevertheless remains true that a collection for public exhibition must be largely explained or interpreted by means of illustrations, models, and descriptive labels. Perhaps the statement of the great British museum administrator,

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Sir William H. Flower, more nearly describes the use and function of a museum, who says: "It is not the objects placed in a museum that constitute its value, so much as the method in which they are displayed and the use made of them for the purpose of education."

Printed labels are the best for permanent display, but as these are expensive the next best are typewritten labels which may be printed on a typewriter having a platen such as is used by the librarians for card catalog work. The ribbon should be black carbon. Where large shells or series of shells illustrating some feature of structure of variation are to be exhibited a uniform black or ivory-colored background may be employed, using a large sheet of dull black paper or a sheet of the bristol board mentioned above for tablets.

Cases. Nearly all molluscan shells are best displayed in horizontal or flat cases. Shelving in an upright case can be used, but this method of installation is not as attractive nor as easy to install as in a flat case. Very large specimens or material preserved in alcohol or other fluid (these should be flat-sided glass jars) are best shown in upright cases or in the A-cases that are now used in many museums. In some museums the space beneath the flat cases is utilized for the purpose of storing the study series in drawers. In some of the older museums these drawers have (or had) glass tops and the contents could be seen by the visitor by simply pulling out the drawer. Excepting where the matter of space is vital this should not be done. The open museum halls are poor places for the proper storage of a research series which must be consulted in the presence of curious visitors who greatly bother the student. These collections should be stored in drawer cabinets kept in rooms especially reserved for research collections and made convenient for their study. This subject is more fully treated on a later page.

For holding cards and labels in an upright position the writer has found the pins and ticket holders sold by stationers to serve the purpose admirably. For attaching specimens to tablets it is better to use wax than glue, the former being easy to remove if the shell is needed for examination, while glue is difficult to remove without injury to the shell. Bright, polished shells, like *Cypreaea* and *Oliva*, are difficult to attach to the tablets on account of their smoothness. The prepared clay known as 'plastene,' 'modelit,' and 'permodello' has been used to some extent by the writer and has been found excellent for this purpose, if the mixture does not have too large a percentage of oil, which discolors the tablet. By drying the clay a trifle the amount of oil may be reduced. This clay usually provides a mold in which the shell may be held in any desired position. The clay is made in several colors among which gray-green, terra cotta, or dark brown are the best. If the shells are of the common kind and are not likely to be needed for study the liquid glues will prove

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the best medium for fastening the specimens to the tablets. The shells may be propped in any desired position until the glue hardens.

Collections for Study

While there are several ways in which a collection of mollusks can be installed for exhibition, there is but one good method of caring for a study or research series of these animals. The dry specimens should be kept in drawer-cabinets. In considering the size of the cabinet the dimensions of the primary unit, the individual tray containing the specimens must first be decided upon.

Pasteboard Trays. These should be made as multiples of the smallest unit. This unit may be 1 x 2 inches for the smallest species and 3 x 2 inches for the larger series, the unit of width here being three inches. If one desires to carry out the 1 x 2 unit for the entire series the larger trays may be multiples of two inches. The various sizes that are the most useful, as learned from experience, are as follows: a. 1 x 2, 2 x 2, 2 x 3, 2 x 4, 4 x 4, 4 x 6, 6 x 6, 6 x 9, 9 x 9, 12 x 12. b. 1 x 3, 2 x 3, 3 x 3, 3 x 4, 3 x 6, 3 x 9, 6 x 6, 9 x 9, 12 x 12. The depth of the trays should be one-half inch, except in the largest size which should be three-fourths of an inch in depth. The trays may be covered with black or white glazed paper which gives them a pleasing appearance. These trays can be made by any box manufacturer. Ingenious students may be able to make their own trays if they have the time. To do this pieces of cardboard should be cut as shown in figure 1 and the four pieces indicated by the dotted line, folded together and attached by adhesive paper. Trays of this kind can be quite economically made in a short time.

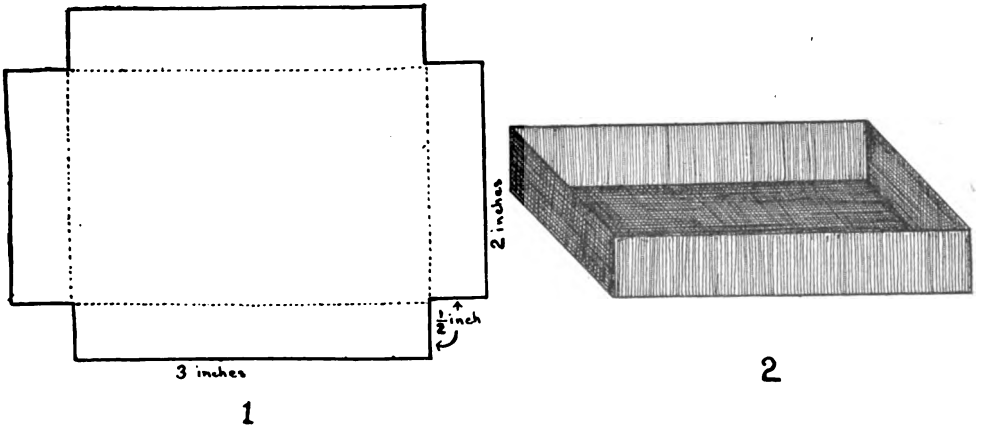

Fig. 1. Method of making a pasteboard tray for holding specimens.

Drawers. Having selected the size of the individual tray the next step is the dimension of the individual drawer. This should be made of a

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size to contain the trays in a manner that there may be no waste space. A convenient size measurement, *inside*, is 15¼ x 21¼ inches. This allows five rows of the three inch unit trays which fit snugly in both dimensions of the drawer. If the two inch is used the drawer should be an inch wider or 16¼ inches. This will hold eight rows of the two-inch unit. The depth of the drawer will depend upon the character of the specimens it will contain. The smallest specimens need a drawer not over an inch in depth while the largest may require a depth of five or six inches. For an all around depth the writer uses a drawer two inches in depth and when larger specimens are installed the space of two drawers is used for one. This method has been found quite satisfactory and does not on the average take more room than when drawers are made of varying depth. It is seldom that specimens of greatly different size will be placed in the same cabinet, if room is wisely left for expansion, as should be done in the larger collections. The drawers mentioned above, which are in use in the University of Illinois Museum, are made of three-eighths inch material for the sides and ends and compo board is used for the bottom. These drawers require no handles and are very inexpensive.

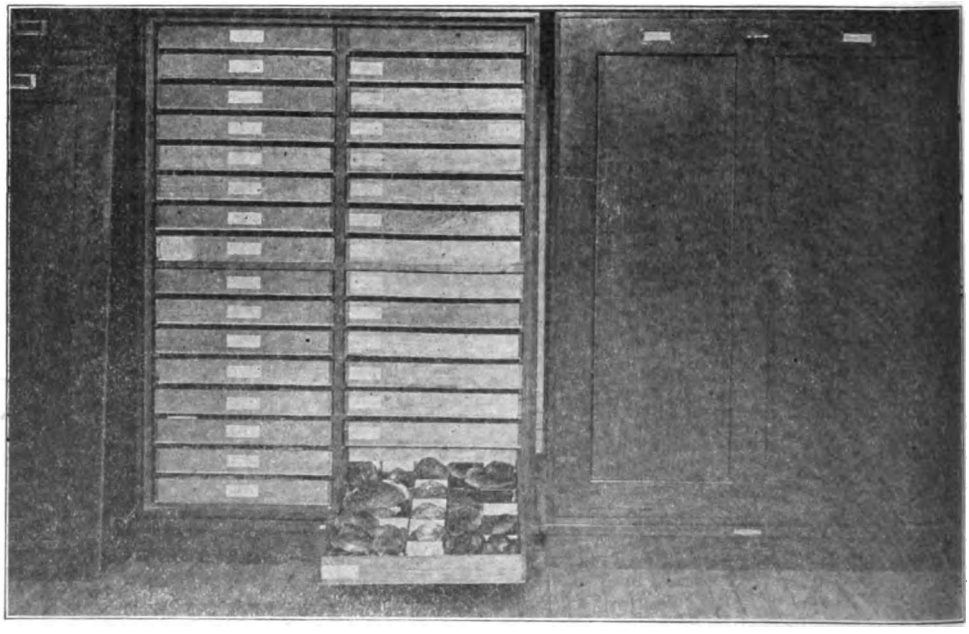

Fig. 2. Storage cabinet of drawers, 32 in each cabinet. An open drawer at the bottom shows the method of attaching labels to the back of the trays, as described in the text. University of Illinois Museum.

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Cabinets. Having determined the sizes of the trays and the drawers the next thing is the size and style of the cabinet to contain the drawers. This should also be of the unit pattern so that several may fit together. The cabinets that are in use in the University of Illinois Museum, and which the writer has used in other museums he has had charge of, are shown in figure 2. These have the following dimensions:

Height 46, width 35½, depth 25 inches, outside measurements.

Drawer space 16⅛ inches wide, 2⅜ inches between the drawer runners.

Drawer runners ⅜ inch pieces sunk in the sides of case 3/16 inch.

Each case holds 32 drawers, 8 in each of four sections.

If the drawers are to be one inch deep the space between the runners should be 1-5/16 inches and the case would hold sixteen drawers in each section or sixty-four in each cabinet. The drawers are really ⅜ of an inch deeper than the dimensions given, this extra space being occupied by the runners. This space allows for extra large shells which may be included with the smaller ones. It is usually essential that all cabinets be of the same size and contain the same size of drawer so that additions and rearrangements may be made without unduly changing the contents of the drawers. This is very important when large additions are made necessitating the rearrangement of a large part of the collection. The drawers may be made of whitewood or basswood and simply shellaced or varnished. The cabinets are best if made of oak and finished in some dark color. The door of the cabinet should be made with a groove which extends entirely around the inner margin. This should fit into a tongue in the sides of the cabinet which also extends entirely around the cabinet. A piece of plush or felt fitted into the groove in the door will keep out the dust very effectually. Rubber has been used but this substance soon loses its resiliency and becomes worthless. The door should be made so that it may be entirely removed from the cabinet so that it will not be in the way when the collection is being studied. The photograph, fig. 2, indicates these points.

For smaller species, as the Pupillidae, Valloniidae, Sphaeriidae, Amnicolidae, as well as many groups of minute marine shells, the writer has used a case made to hold legal blanks which has proved very convenient and satisfactory. The dimensions of the drawers are:

Length 14¼, width 9, depth 1 inch; height of case of ten drawers 14 inches.

Each drawer holds 56 of the 1 x 2 inch unit trays or 560 trays in the cabinet. These cases are admirably adapted for holding these small shells, the drawers not being large enough to be cumbersome as is the case with a large drawer filled with these small trays. Several legal blank cases may be installed in one of the larger cabinets if it is desired to keep the cabinets perfectly uniform. The only possible criticism may be that

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these legal blank cases are not perfectly dust proof and for permanent installation they should, perhaps, be enclosed in a cabinet as suggested above. Each drawer should be labelled with the name of the contents and each cabinet should have the name of the group it contains.

Bottles and Vials. For the safety of the collections glass bottles or vials should be provided for all shells under ¾ inch diameter. These should be made in different diameters but only of two lengths to fit the two unit widths of trays, two and three inches wide. Convenient sizes are as follows:

1¾ x ⅜, 1¾ x ½, 1¾ x ¾, 1¾ x 1 inch.
2½ x ¾, 2½ x ½, 2½ x ¾, 2½ x 1 inch.

Occasionally a larger size will be needed and a vial of 1½ inch diameter will be found useful. Only a few of this size will usually be required. The bottles known as shell vials, obtainable through almost any druggist, are especially adapted for the preservation of molluscan material and can be made of any of the sizes mentioned above. Short corks may be used but these should be rolled or squeezed to soften them so that the fragile tubes may not be broken when the cork is forced into the mouth of the vial. Rolling with a hard piece of wood or metal or pressing between a pair of large flat-nosed pliers will be found to accomplish this purpose admirably. Homeopathic vials may be used but these are not as good for dry specimens as the shell vials. Where expense is a serious item very good containers may be made by rolling a piece of stiff paper over a lead pencil or other round object the size of the required container and then gluing or pasting the edges together and closing one end with cotton. A cotton cork may also be used for the other end. The modus operandi of this method is indicated in figure 3. The cylinders may be made in lengths of legal blanks and then cut off in lengths to fit the trays.

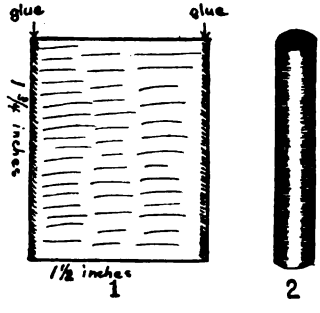

Fig. 3. Method of making paper shell tubes for holding small specimens.

Storage of Alcoholic Material

The proper storage of material preserved in alcohol is a matter requiring considerable attention. For this purpose homeopathic vials are better

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than shell vials, because they are thicker and the strong, reinforced opening makes it possible to press a cork in very tightly, which retards evaporation of the liquid contents. Fairly large vials should be used even for small specimens in order that the storage may be uniform and liquid enough provided for the specimens to obviate frequent filling of the containers. Several sizes of bottles, 2, 4, 6 ounce, will be required to preserve the larger specimens. For mussels or series of specimens the clamp-top, all glass fruit jars (Atlas for example) are excellent for storage purposes. The old-fashioned Mason jar is not good because the liquid evaporates quickly and the metal screw top cannot be made permanently tight, besides becoming very unsightly in a short time. All of the jars should be of glass, except the rubbers, and these will need to be changed at intervals. The quart and pint jars have been found the most useful for purposes of storage, although the two quart size may be necessary at times.

Professor Frank Smith, of the Department of Zoology, University of Illinois, has made use of a method, first devised by the United States National Museum, for the preservation and storage of small specimens in vials which has much to commend it. The small vials, after being filled with alcohol or other preservative and having a wad of cotton placed in the mouth of the vial, are stored, bottom upward, in a large jar, of two liter capacity or larger, which is then filled with alcohol or other fluid. By this means the smaller vials may be kept without adding new liquid for a long time. Also, the large jar will become empty before the small vials and thus a warning is given before any damage can be done to the specimen. It often happens when valuable material is stored in many small vials that lack of proper attention permits the vials to become empty of fluid and the specimens dry out and are thus ruined.

Many zoologists will prefer the single bottle method, however, on account of the accessibility of the material, and for such the storage should be in standard racks, which may be stored on compo board shelves in the unit cabinets described previously. These racks will vary in width but should be of the same length. Convenient dimensions are as follows:

Vials, length 22, width 1⅜, height side 2¼, height front 3 inches.

Bottles, length 22, width 2¼, height side 2¼, height front 3 inches.

The general form used is indicated in figure 4. The stock should be

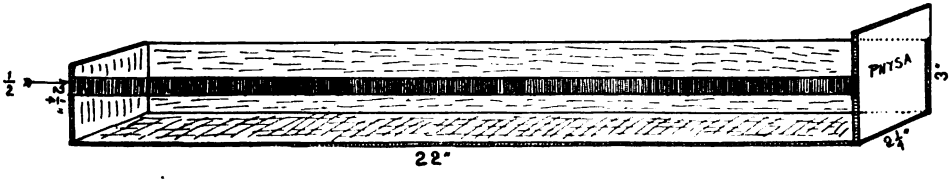

Fig. 4. Standard rack for holding alcoholic material, vial size.

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3/8 inch for ends and 3/16 inch for bottom and sides. The large jars are perhaps best stored on shelves, although a rack similar to the one suggested for the vials and bottles, but made large enough to hold the jars, may be used. These racks may be made by any good carpenter or the lumber can be cut in a mill and the collector can put the racks together himself.

Registration and Labeling

Every set of specimens should have with it a label giving the name of the species, its locality, the principal ecological conditions under which it was found, the name of the collector, and the name of the authority who determined the species, as well as the date of collection. For this purpose cardboard labels just the width of the inside of the unit tray, 1 x 2 or 1 x 3 inches, may be used. The writer has found by experience that an excellent method of attaching the label to the tray is to glue the upper edge of the label to the upper margin of the unit width of the tray, at the back. When a whole drawer is arranged with the labels affixed in this manner the different species and their localities may easily be read. The specimens or vial of specimens lie in front of this label, as shown in figure 2. Genera or group divisions may be indicated on labels fastened to the bottom of the 1 x 2 or 1 x 3 inch trays. A sample of label in use in the Museum of the University of Illinois is shown below.

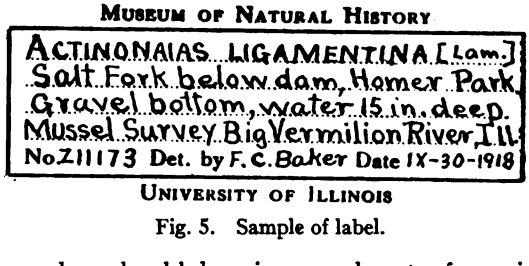

Fig. 5. Sample of label.

A catalog number should be given each set of specimens and this number should be placed in the vial containing the specimens or in the case of large specimens, written on the shell. For mussels both valves should be numbered. The best quality of indelible carbon ink should be used for this purpose. The writer has found Higgin's eternal ink (waterproof) to be the best for all purposes. The alcoholic material should have a label placed in each bottle written with the same kind of ink. Cardboard labels for this purpose are good. A permanent cloth known as 'mapstock' velum, sold by Jos. Bancroft & Sons Co., Rockford near Wilmington, Delaware, has been found admirable for this purpose.

A serial catalog kept in a book and a card catalog are invaluable for the proper recording and convenient classification of a collection. The book should be arranged to contain the serial numbers of the collection.

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This volume may be made as elaborate as the pocket book of the collector will permit, varying from a simple note book to a large printed folio. For museums and large collections of private individuals the large folio is by all means the best. This may be arranged with the headings suggested below.

Cat. No.	Name	Locality	No. of specimens	Received from	Collected by	Date	Remarks

Other entries, such as original no., identified by, dry, alcoholic, etc., may be used if it is desired to elaborate further. For large institutions an accession catalog is necessary, in which is recorded the material by lots as received. In such cases an entry, accession number, is usually made room for after catalog number in the species catalog.

The card catalog should contain the references to all of the lots of one species, showing the different places from which they came, on one card, or each lot of a species may have all of the information recorded on one card. The first is more convenient for a small collection but the latter is perhaps better for a large institution or collection, giving all of the known data concerning each species lot on one card. The cards should be arranged alphabetically under the genera, the names of which should appear on guide cards, as is done for library card catalogs. Experience will suggest many ways in which the cataloging may be so arranged as to make the collection most useful, which is its legitimate function.

In closing let me say that a collection of mollusks is valuable principally for the information which it may contain. It is of paramount importance, therefore, that the data or information concerning each lot of specimens be made as accurate and complete as possible. This should be done in the field if possible and not left until later when memory may play one tricks as to the exact habitat of some specimens in a large lot. There are many questions still unsettled regarding the classification, geographic distribution, ecological habitat, and economic importance of this class of animals and any conscientious collector may add real scientific knowledge concerning some common species by exercising care and intelligence in making collections.

Some Papers Relating to the Collecting and Preparation of Mollusca for Both Exhibition and Study

Baker, Frank C.

1898. Mollusca of the Chicago Area. The Pelecypoda. Section VI, Instructions for Collecting Mollusks, pp. 25–32.

1900. A new Museum Tablet. Amer. Nat., XXXIV, pp. 283–284.

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1902. The Descriptive Arrangement of Museum Collections. The Museums Journal (English), II, pp. 106–110.

1904. The Arrangement of the Collection of Mollusca in the Chicago Academy of Sciences. Museums Journal (English), II, pp. 354–360.

1909. Suggestions for an Educational Exhibit of Mollusks. Proc. Amer. Assoc. Museums, III, pp. 56–59.

1910. Same title, Museums Journal, IX, pp. 394–397.

DALL, WILLIAM H.

1892. Instructions for Collecting Mollusks and other Useful Hints for the Conchologist. Bull. U. S. Nat. Museum, No. 39, Part G, pp. 1–56.

STERKI, VICTOR.

1916. Some Directions and Suggestions for Collecting the Sphaeriidae and Aquatic Gastropods. Annals Carnegie Museum, X, pp. 478–486.

WALKER, BRYANT.

1902. Hints on Collecting Land and Fresh-water Mollusca. Journ. of Applied Microscopy and Laboratory Methods, V, No. 9, pp. 1954–1961.

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PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

Minutes of the Chicago Meeting

The thirty-ninth annual meeting of the American Microscopical Society was held in affiliation with the A.A.A.S. at Chicago, Ill., Dec. 29, 1920.

In the absence of President Galloway, Vice-President Juday acted as chairman.

The report of the Treasurer for the year 1920 was read by the Secretary and was referred to an auditing committee composed of Professors F. H. Krecker and J. W. Kostir.

The report of the Custodian was read by the Secretary and referred to an auditing committee composed of Messrs. Edw. Pennock and Edw. P. Dolbey. The meeting voted unanimously to send greetings to the Custodian, Mr. Magnus Pflaum, and to congratulate him on the growth of the Spencer-Tolles fund.

The Secretary presented a general report on the affairs of his office.

The following officers were nominated and elected for the constitutional periods: President, Professor Frank Smith, University of Illinois; First Vice-President, Professor J. E. Ackert, Kansas State Agricultural College; Second Vice-President, Professor Ruth Marshall, Rockford College.

Professor E. M. Gilbert, University of Wisconsin, Dr. B. H. Ransom, Bureau of Animal Industry, Professor Chancey Juday, University of Wisconsin, were chosen as the elective members of the Executive Committee for 1921.

Adjourned. Paul S. Welch, *Secretary*

CUSTODIAN'S REPORT FOR THE YEAR 1920

SPENCER-TOLLES FUND

Balance reported for the year 1919			$5,958.93
May 17, Sale of Transactions		$ 150.00	
June 30, Dividend		238.32	
Dec. 6, Sale of Transactions		80.00	
Dec. 31, Dividends		190.41	658.73
Net amount invested			6,617.66

Increase during the year $658.73.

TOTALS

Receipts

All contributions		800.27	
All Sales of Transactions		1,108.38	
All Life-members		300.00	
All interest and dividends		4,699.01	
		6,907.66	

Disbursements

All grants	250.00		
All dues for life-members	40.00	290.00	6,617.66

Life-members: (Robert Brown, dec'd); J. Stanford Brown; Seth Bunker Capp; Harry B. Dunkanson; A. H. Elliott; John Hately.

Contributions of $50 and over: John Aspinwal; Iron City Microscopical Society; Magnus Pflaum; Troy Scientific Society.

Magnus Pflaum,
Custodian.
Philadelphia, Pa., Jan. 15, 1921.

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The undersigned having examined the foregoing report certify that we have found the same true and correct.

EDWARD PENNOCK,
EDW. P. DOLBEY,
Auditing Committee.

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY DECEMBER 24, 1919 TO DECEMBER 17, 1920

RECEIPTS

Balance on hand, December 24, 1919	$ 480.04
Dues received for Volume 38 or before	52.10
Dues received for Volume 39	186.00
Dues received for Volume 40	272.10
Initiation fees	90.00
Subscriptions for Volume 38 or before	38.00
Subscriptions for Volume 39	193.90
Subscriptions for Volume 40	28.80
Sales of Transactions, duplicates and back numbers	325.70
Advertising, Volume 38	192.50
Advertising, Volume 39	14.00
Grant from Spencer-Tolles Fund	100.00
Messrs. Anderson and Walker, plates	76.15
Mr. A. Jacot, plates	60.00
Sundries	3.12
TOTAL	$2,112.41

EXPENDITURES

Printing Transactions, Volume 38, No. 4	$ 152.75
Plates for Volume 38, No. 4	23.00
Printing Transactions, Volume 39, No. 1	397.91
Printing Transactions, Volume 39, No. 2	262.51
Printing Transactions, Volume 39, No. 3	243.82
Plates for Volume 39, No. 3	116.30
Printing Authors' Reprints	45.90
Postage and Express for Secretary	43.16
Postage and Express for Treasurer	8.77
Office expenses of Secretary	56.46
Office expenses of Treasurer	32.64
Office expenses of Custodian	5.00
Secretary, Trip to St. Louis Meeting	50.00
Spencer-Tolles Fund	230.00
Balance on hand	444.19
TOTAL CREDITS	$2,112.41

W. F. HENDERSON, *Treasurer.*

The accounts of W. F. Henderson, Treasurer of the American Microscopical Society, for the year of Dec. 24, 1919, to Dec. 17, 1920, have been examined by the Auditing Committee and found to be correct.

Respectfully submitted,
F. H. KRECKER,
W. J. KOSTIR,
Auditing Committee.

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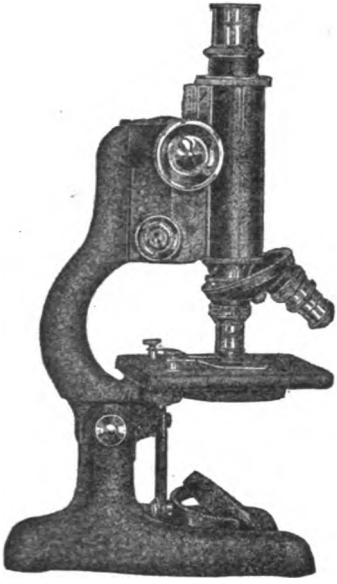

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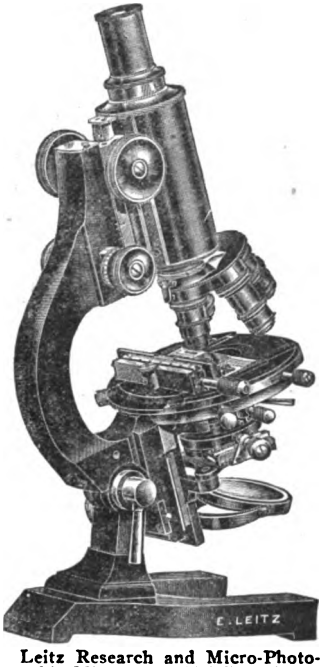

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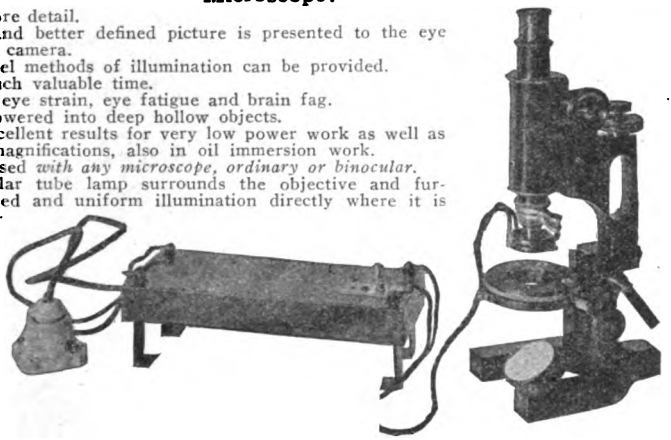

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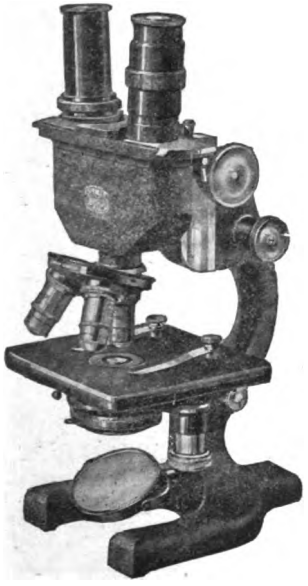

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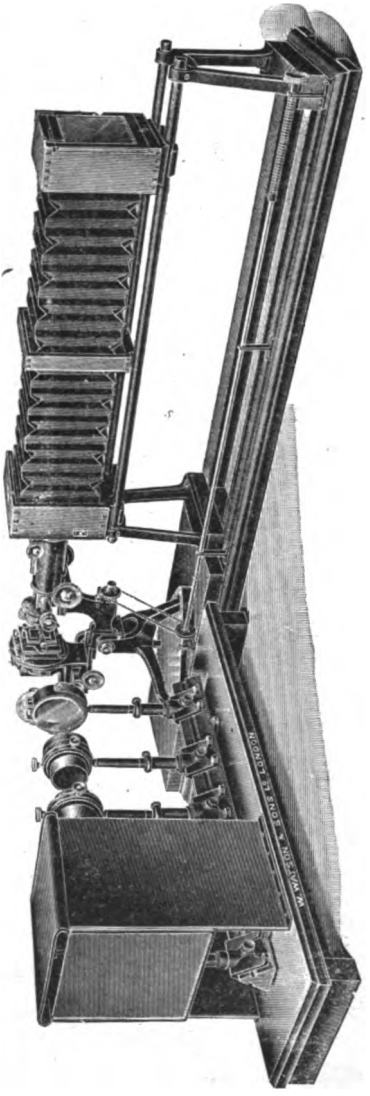

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INDEX TO ADVERTISEMENTS

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TABLE OF CONTENTS

For Volume XL, Number 2, April, 1921

Larval Flukes from Georgia, with two plates, by E. C. Faust 49

On the Nature of Structures Characteristic of Cnidosporidian Spores, by R. Kudo.... 59

Department of Summaries

Recent Advances in Parasitology, by E. C. Faust 75

Department of Methods, Reviews, Abstracts, and Briefer Articles

A Method for Orienting and Mounting Microscopical Objects in Glycerine, by Charles Bullard 89

A Method of Demonstrating the Sheath Structure of a Desmid, with one figure, by W. R. Taylor 94

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)
Vol. XL APRIL, 1921 No. 2

LARVAL FLUKES FROM GEORGIA[1]

(With two Plates)

By
ERNEST CARROLL FAUST
Parasitologist, Union Medical College, Peking

In a previous study (Faust 1919) I have reviewed the cercariae described from the United States. I discussed the regions from which forms had been reported and suggested that the Southeastern United States offered an unexplored field where conditions were eminently favorable for their existence.

From October 1918 to May 1919 I had the opportunity to examine several hundred mollusks from the region of Rome, Georgia, and in two of these species, *Goniobasis carinifera* Lamarck and *Anculosa carinata* Brug, I have discovered the larval flukes described in this paper. I wish to acknowledge my indebtedness to Mrs. Lola Swift Faust for the collection of the material and to Mr. Bryant Walker for the determination of the hosts.

The larvae represented in this investigation are all distomes and fall into the following groups:

Allocreadiine larvae	1
xiphidiocercariae	3
microcercous cercaria	1
echinostome larva	1
furcocercariae	2
cystocercous cercaria	1

All of these forms are new to this region. Two have been described in a previous paper (Faust 1919a).

Cercaria thalia nov. spec.

(Figs. 1, 2)

Host: *Goniobasis carinifera* Lamarck.

This interesting larva for which I propose the name *Cercaria thalia* was found in *Goniobasis carinifera* Lamarck, collected from Rotary Lake,

[1] Contributions from the Department of Pathology, Union Medical College, Peking, China.

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Rome, Georgia. The redia and cercaria stages were dissected out of the digestive gland of the snail, while the agamodistomes were found in the lung sac. Both the redia and the cercaria were characterized by a graceful movement.

In the cercaria a good share of the organs are rendered opalescent by the sub-integumentary cells which are muciferous rather than cystogenous in structure. The larva is distinguished by a pair of eye-spots just lateral to the pharynx. The body measures 0.4 mm. in length by 0.15 mm. in width. The tail is slightly longer and has a proximal diameter of 57μ. The oral sucker measures 71μ in section while the acetabulum when extended has a diameter of 90μ. No spines have been observed on the body or tail. There is a prepharynx and a small pharynx with a short esophagus showing constrictions at intervals along its course. The ceca terminate about one-third the body distance from the posterior end. There are three pairs of mucin glands, with ducts opening on the dorsal aspect of the orifice.

The excretory bladder has a long median shank and slender cornua so that the entire appearance is that of a furculum with a supporting stem. I have observed a triplet group of flame cells at the anterior extremity of the body and a similar number at the posterior limit, but have been unable to make out the number of groups intermediate. From the group formula which I have previously shown to exist for this family of trematodes (Faust 1919a:334) four other flame cell units are to be expected. A median collecting tubule runs almost the entire distance of the tail.

The agamodistome (Fig. 2) lacks the pigmented eyespots and, indeed, shows few of the larval characters. The most readily recognizable common feature is the excretory bladder. On the other hand this fluke shows evidences of rapid maturity. The shape is that of a mature worm rather than that of a larva. The ceca have enlarged and what is especially noteworthy, the genital organs have reached a high degree of complexity. The testes and ovary are in their relative positions, the vitelline follic'es are well formed, the seminal vesicle and the seminal receptacle are each conspicuous and Laurer's canal is distinguishable So well developed are all of these organs that it is a simple matter to place the worm in the Allocreadiinae. Thus a considerable share of the life history of this animal is at hand, even tho only the first larval host is known.

The cercaria is produced in a redia with a small orange-colored gut, a short prepharyngeal region, a small pharynx, and a small but conspicuous birth pore. Moreover, both daughter rediae and cercariae develop within the same parent redia. It is noteworthy that the cercaria possesses eyespots, a common feature of the larval allocreadid form, but lacks a stylet.

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Cercaria camilla nov. spec.
(Fig. 3)

Host: *Goniobasis carinifera* Lamarck.

The stylet cercaria for which I propose the name *Cercaria camilla* is a rapidly moving larva with an oblong-ovate body 0.16 mm. long by 0.066 mm. wide and a tail 0.11 mm. long and 18μ in diameter at the base. Both body and tail are spinose. The oral sucker is 26μ in diameter while the acetabulum measures only 13μ in transection. The latter lies midway between the anterior and posterior limits of the body.

Inserted into the roof of the anterior end is a typical quilled stylet. The oral sucker leads into an enormous prepharyngeal pocket 53μ in diameter, with a thick, semi-muscular, semi-mucoid wall. This in turn leads directly into a minute muscular pharynx. Beyond the pharynx a short esophagus connects with a part of short blunt ceca. The mucin glands consist of three pairs. Two of these pairs are granular, acidophilic, while one pair is densely muciferous, basophilic in reaction. The contents of each gland passes thru a long duct to empty at the side of the stylet.

The excretory bladder has the shape of an inverted truncated pyramid, from which emerge delicate collecting tubules. These tubules when traced to their sources reveal on each side of the body four pairs of flame cells posteriorly disposed. A single collecting tubule runs down the middle part of the tail. The flame-cell formula has an identical common denominator with that of *Allocreadium isoporum* Looss (Faust 1919a:327, 334), namely

$$(2 \quad 2+2+2) \quad (2+2)$$

The parthenita of *C. camilla* is a very simple sac-shaped sporocyst containing eight to twelve cercariae. Encystment of the cercariae has not been observed.

Cercaria tabitha nov. spec.
(Fig. 4)

Host: *Goniobasis carinifera* Lamarck.

This cercaria for which I suggest the name *Cercaria tabitha* has an ovoid body 0.15 mm. long by 0.088 mm. wide and a blunt tail 0.1 mm. long by 0.017 mm. in diameter at the base. The body is covered with heavy spines and the oral sucker is provided with a blunt stylet. The large oral sucker, 32μ in diameter, is provided internally with a thick mucoid substance resembling that of the stylet. Behind this is a small pharynx. There are four mucin glands on each side of the body, one of which contains a basophilic substance. They all empty far laterad at the margin of the oral sucker. The remainder of the digestive tract has not been traced. The acetabulum is in the middle of the ventral side of the body. It measures 21μ in diameter.

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The excretory bladder is cup-shaped. The collecting tubules emerge from the anterolateral aspect of the bladder while the pore is posteriad.

The sporocyst is a simple, sacculate structure, containing up to twenty-four larvae.

The tail of the cercaria is dropped readily under a cover glass, but encystment has not been observed.

Cercaria pandora nov. spec.
(Fig. 8)

Host: *Goniobasis carinifera* Lamarck.

This little larva for which I suggest the name *Cercaria pandora* is oblong-ovate with a body measurement of 0.145 mm. in length by 0.054 mm. in width and a tail not more than half as long by 17μ in diameter at the base. The anterior sucker is large, measuring 38μ in diameter. The acetabulum is mesad and very small (14μ). A small stylet with a sharp, delicate point is inserted into the dorsal wall of the oral sucker. A small pharynx is located just behind the orifice. It leads into a forked gut densely surrounded with gland cells. The mucin glands consist of four pairs, the posterior one of which has a large nucleus and gives an acidophilic reaction.

The excretory bladder is a roughly truncated cone with conspicuous lateral cornua. The caudal excretory canal has several tributaries but no observable flame cells.

The larva develops in large numbers in simple sacculate sporocysts.

Cercaria medea nov. spec.
(Fig. 7)

Host: *Goniobasis carinifera* Lamarck.

This larval fluke for which I suggest the name *Cercaria medea* has a long, slender body and a short, stubby tail. The latter is so limited in extent as to place the larva in the group of the microcercous cercariae.

The animal measures 0.22 mm. in length by 0.065 mm. in width and has a tail only 21μ long. The latter structure in semiglandular, the products of which are poured into a common atrium. The cells are chromophobic and have irregular shaped nuclei. The anteriormost part of the body together with the acetabulum bear small sharp spines. The oral sucker has a diameter of 25μ and the acetabulum of 27μ. Inserted in the dorsal wall of the oral sucker is a minute, simple-pointed stylet (See fig. 7a).

Behind the oral sucker is a small pharynx. A long, narrow esophagus runs back from this to the anterior aspect of the acetabulum, whence the ceca continue posteriad to the subcaudal region of the body. A group of twelve to fifteen mucin glands is situated on each side of the body posterior

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to the acetabulum. A very delicate bundle of ducts conveys the products of these glands to the region of the stylet.

The excretory bladder is long and bag-shaped, giving rise to a pair of cornua laterad just behind the acetabulum. The main collecting tubule on each side of the body bifurcates just anteriad to the acetabulum. A median tubule extends into the tail and opens into the mucin pocket.

Two strings of germ cells extend longitudinally across the acetabulum.

The sporocyst in which the cercariae develop varies greatly in size. It has a muscular anterior end and at irregular intervals has constrictions. The movement of both the cercaria and the sporocyst is slow.

Cercaria penthesilia nov. spec.
(Fig. 9)

Host: *Goniobasis carinifera* Lamarck.

This cercaria for which I propose the name *Cercaria penthesilia* is an echinostome larva which is probably immature, in which the circlet of collar spines has not yet developed. It measures 0.2 mm. in length by 0.084 mm. in width, and has a tail 0.135 mm. long by 14μ in diameter at the base. The body is covered with many short spines closely studded together. The oral sucker has a diameter of 30μ and the acetabulum of 32μ. The entire body has a thick subintegumentary lining of long rhabditiform cystogenous granules, which bear evidence of the animal's future encystment. A fluted keel is found in the distal third of the tail.

There is a short prepharyngeal region of the digestive tube followed by a small pharynx. The esophagus forks almost immediately to form ceca which extend far caudad. Paired groups of acidophilic mucin glands run mesad to the ceca.

The excretory bladder is spheroidal with a large opening dorsocaudad. The cornu on each side is quite inconspicuous. From it a dilated collecting tube is traced which becomes narrow in the region of the pharynx. Several flame cells have been found but the exact number has not been worked out. Running posteriad from the bladder is a collecting tube for the tail. Halfway down the tail it divides to form a pair of tubules opening laterad.

The genital system is represented by two groups of germ cells lying longitudinally across the acetabulum. The nervous system is conspicuous, with especially large ventral trunks.

The redia in which the cercaria develops is provided with the pharynx, gut, birthpore and lateral appendages found in the echinostome group.

Cercaria quattuor-solenata Faust 1919
and
Cercaria furcicauda Faust 1919
(Figs. 5, 10)

These furcicaudous species, *Cercaria quattuor-solenata*, and *C. furcicauda*, were originally described in connection with a study of the excretory

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system of distome cercariae (Faust 1919a: 337, 338) and are included here for the sake of completeness. The host of both species is *Anculosa carinata* Brug.

Cercaria stephanocauda nov. spec.
(Fi-. 6)

This interesting larva which I have designated as *Cercaria stephanocauda* represents a group which has recently received considerable attention (Ward 1916, Pratt 1919, Faust 1918). Five species have been described from North America. While in many respects the structure of the body of the immature larva resembles that of the described species, the tail is unique.

The worm has a body 2 mm. long and 1.2 mm. wide. The shank of the tail is 4 mm. long, while the lamellate furcae measure 1.1 mm. long and 0.5 mm. wide. The anterior fourth of the tail shank consists of a collared region with about nine definite ringed constrictions, running around the tail. At the posterior end of this collar there are numerous tubercules in a single row which are the only traces of mammilations anywhere on the body. Behind this collar the tail proceeds toward the distal portion with gradual constriction. The tail is attached to the body proximally by a number of powerful longitudinal muscles.

The oral sucker measures 430μ in diameter and the smaller ventral sucker, 360μ. The pharynx just behind the oral sucker has a diameter of 200μ. It connects with the ceca by a very short esophagus. The ceca proceed directly laterad almost to the posterior margin of the body. They are slightly convoluted.

The excretory system consists of a minute bladder and large collecting tubules. The latter reach to the region of the oral sucker, then are reflexed and break up into capillaries. A large collecting tubule extends the length of the tail, forking into the furcae and opening outward at the distal end of each furca.

The genital glands are very immature as contrasted with the condition in *Cercaria macrostoma* (Faust 1918). The germ glands are connected by a chain of cells which lie in the median line, with one gland offside to the left.

The sporocyst is large, being a simple sac with undifferentiated tissue.

Summary

A study of cercariae taken from snails at Rome, Georgia, shows new species with interesting relations to previously described forms.

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Description of Plates

Figs. 1, 2.—*Cercaria thalia* 1, cercaria, ventral view, X 126; 2, agamodistome, ventral view, showing precocious development of genital organs, X 40.

Fig. 3.—*Cercaria camilla*, ventral view, X 400.

Fig. 4.—*Cercaria tabitha*, ventral view, X 285.

Fig. 5.—*Cercaria quattuor-solenata*, ventral view, X 200.

Fig. 6.—*Cercaria stephanocauda*, ventral view, X 28.

Fig. 7.—*Cercaria medea*, ventral view, X 90; 7a, detail of stylet.

Fig. 8.—*Cercaria pandora*, ventral view, X 285.

Fig. 9.—*Cercaria penthesilia*, ventral view, X 276.

Fig. 10.—*Cercaria furcicauda*, ventral view, X 200.

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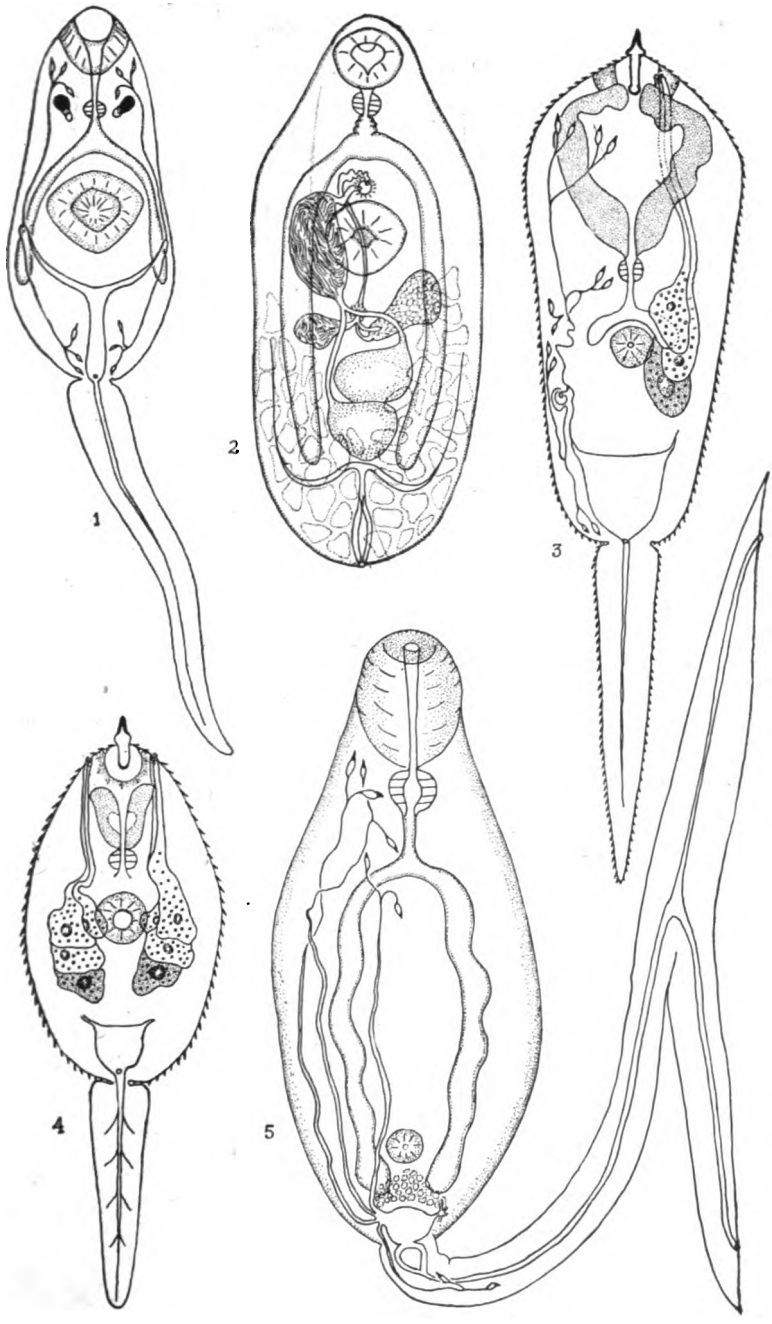

PLATE II.

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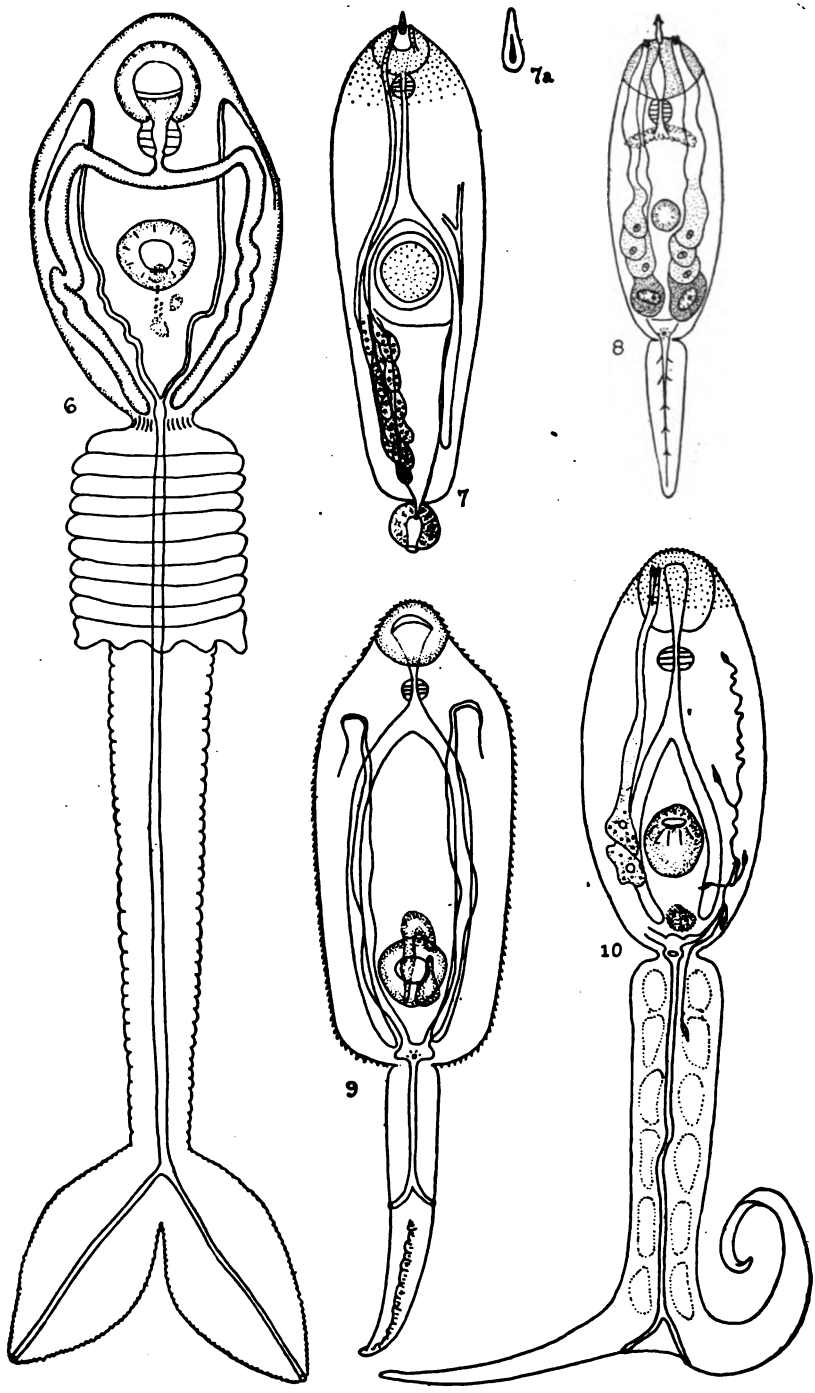

PLATE III.

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ON THE NATURE OF STRUCTURES CHARACTERISTIC OF CNIDOSPORIDIAN SPORES[1]

By

R. Kudo

CONTENTS

Introduction 59
Material and methods 60
The spore membrane 60
The polar filament 65
The iodinophilous vacuole 70
Summary 73
Bibliography 73

Introduction

Although our knowledge of the morphology and development of Cnidosporidia has recently been greatly increased, little is known about the chemical nature of the different parts which compose the spores. In the study of artificial cultivation of Cnidosporidia, it becomes necessary to obtain a definite view regarding this point. Unfortunately the majority of opinions advanced by several authors are not accompanied by any definite experimental data. This is possibly due to the fact that because the organisms have never been found in direct contact with higher vertebrates as their parasites, they have not interested such a large number of investigators as did other parasitic Protozoa which are directly responsible for serious diseases among mammals, and that the number of organisms found at one time is not generally large so that only little is left when the study of their morphology and development is completed.

Erdmann (1917:317–318) has recently expressed a view that the polar filaments of the spore of *Chloromyxum leydigi* were probably composed of glycogen and plastin, and further suggested, in referring to my paper on the polar filament of the spore of *Nosema bombycis* (Kudo, 1913), that her method might be useful for the study of the nature of polar filaments of the Microsporidian.

I have been working for some time on the subject by using several species of Microsporidia and Myxosporidia. The results thus far obtained in regard to the polar filaments are entirely different from those of Erdmann's. Besides, as I believe the results of observations upon the nature of spore membrane and the so-called iodinophilous vacuole seem to be

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 182.

[2] I am greatly indebted to Professor Henry B. Ward who kindly placed the material at my disposal.

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more or less interesting, they are summarized and presented in the following pages.

Material and Methods

For the study of spore membrane of Myxosporidia, *Henneguya salminicola* Ward[2] was chosen. This species, as was described by Ward (1920), forms numerous large cysts in the host tissue. Moreover, it was not only obtainable in a very large number, but was also favorable for the study due to the presence of the posterior process characteristic of the genus. The Myxosporidian had been preserved in formol since 1914 together with the host tissue. The cysts were isolated, washed thoroughly with distilled water, and the cyst wall was punctured. The emulsion thus obtained was used to make numerous smears. For Microsporidia, spores of *Nosema bombycis* Nägeli and *Nosema apis* Zander were selected. Although they are much smaller than such a form as *Thelohania magna* Kudo (Kudo, 1920), the enormous number that could be procured favored their selection as representatives of Microsporidia. The infected silk-worm moths and the infected ventriculus and intestine of honey bees were emulsified with distilled water, and were used as stock emulsions.

The nature of the polar filaments was studied by using the fresh spores of *Myxobolus mesentericus* Kudo, *Mitraspora elongata* Kudo, *Leptotheca ohlmacheri* (Gurley) Labbé, *Nosema bombycis*, *Nosema apis* and *Thelohania magna*.

The following four species of the family Myxobolidae were used for the study of iodinophilous vacuole. They were fixed either in formol or sublimate alcohol as are indicated below: *Myxobolus discrepans* Kudo and *Myxobolus mesentericus* Kudo fixed in sublimate alcohol, and preserved in 95% alcohol; *Myxobolus aureatus* Ward and *Henneguya salminicola* Ward fixed and preserved in formol. Besides, the following five species from other genera were also studied for the comparison: *Wardia ovinocua* Kudo, *Mitraspora elongata* Kudo, *Leptotheca ohlmacheri* (Gurley) Labbé, *Chloromyxum wardi* Kudo and *Myxidium americanum* Kudo.

The experiments were conducted both in smears and section preparations. In former case, the smears were first allowed to dry before the application of chemicals or stains especially for the determination of spore membrane. Detailed descriptions of methods used will be given in the corresponding chapters.

The Spore Membrane

The strong resistance of spores of *Nosema bombycis* against certain chemicals has long been known by the studies of Frei and Lebert (1856) and Haberlandt and Verson (1870), as the parasite is the cause of the well known pebrine disease of silk worms. These authors, however, attacked

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the problem with the aim of destroying the spores rather than the determination of its nature.

As to the spore membrane of Myxosporidia, Bütschli seems to be the first who studied the effect of concentrated sulphuric acid upon the spores of *Myxobolus mülleri* Bütschli. He (Bütschli, 1881:634) states as follows: "Die Schalensubstanz besitzt eine recht ansehnliche Widerstands-fähigkeit gegen Reagentien; dennoch ist die Angabe Balbiani's, dass sie auch in erhitzten Mineralsäuren sich erhalte, nicht begründet. Erstmaliges Erhitzen in koncentrirter Schwefelsäure liess zwar die Schalen nur in ihre beiden Klappen zerfallen, zerstörte dagegen die sogleich zu erwähnenden Polkapseln völlig; nochmaliges Erhitzen bewirkt jedoch auch völlige Zerstörung der Schalen." Balbiani (1883:202) on the other hand states that the boiling sulphuric acid does not affect the Myxosporidian spore membrane. Gurley (1894:83) agreed with Bütschli, writing as follows: "This (shell) substance is thin, very transparent, insoluble in the strongest acids and alkalies in the cold, certainly in some, and probably in most species destroyed by (soluble in ?) concentrated sulphuric acid at its boiling temperature."

It is my opinion that the controversy of the results of experiments among these authors are probably due to the difference in the concentration of the acid used. Although none of the investigators mentioned their exact technique, it is almost certain that they added concentrated sulphuric acid to the emulsion of the spores they had. Consequently they did not observe the effect of the truly concentrated sulphuric acid, but that of more or less diluted acid which varied from a stronger concentration (Bütschli and Gurley) to a weaker one (Balbiani) according to the relative amounts of water and the acid. In order to avoid this error in the present experiments, the smears of spore emulsions were dried on the slides before they were subjected to the action of reagents.

None of the above mentioned three authors, however, has expressed an opinion in regard to the chemical nature of the membrane. It was Thélohan who advanced his observations concerning this point. Yet in his valuable work on Myxosporidia, Thélohan (1895:260) simply states as follows: "Je n'ai pas déterminé la nature chimique de la substance qui constitue l'enveloppe. Elle ne présente en tout cas aucun des charactères de la cellulose."

Although a large number of papers on Cnidosporidia has appeared lately, none touches this problem. Davis (1917:210) states that "surrounding the spore is a thin, tough, transparent membrane, the sporocyst, which is probably of a chitinoid nature.' He, however, does not give any experimental datum to support this statement. Auerbach (1910:17) in his monograph wrote negatively as follows: "Die chemische Zusammensetzung der Schale ist meines Wissens noch nicht sicher bekannt."

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Thus the opinions of a few investigators regarding the chemical nature of the Cnidosporidian spore membrane may be summarized as follows: The spore membrane of Myxosporidia does not give a positive cellulose reaction, and seems to be of chitinoid nature.

The results of my experiments will be reported here.

a) Tests for albuminoid substances:—Spores of *Henneguya salminicola*, *Nosema bombycis* and *Nosema spis* are not affected by boiling potassium hydrate solution (35 per cent), and do not give any recognizable positive Millon's reaction. It may therefore be said that the spore membrane is not composed of albuminoid substances.

b) Tests for cellulose:—For the control of the cellulose tests, a filter paper was used.

1) Ammoniacal solution of copper oxide. Six fibers of the filter paper were taken out, and were mounted on two slides, each containing three fibers. To one distilled water was added, while to the other ammoniacal solution of copper oxide. Both were covered with cover glasses. The water emulsions of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* were smeared on slides, were dried, and were treated in the same way as the fibers of filter paper. The preparations were kept in a moist chamber. The results of observations are as follows:

	Soon after the treatment	16 hours later	36 hours later
Cellulose with water	Outline of fibers sharp	No change	No change
Cellulose with ammon. solution of copper oxide	Outline of fibers less sharp	Outline faint	More invisible
Henneguya salm., *Nosema bombycis* and *N. apis* with water	Outline of spores sharp	No change	No change
Henn. salminicola, *Nosema apis*, and *N. bombycis* with amm. sol. of copper oxide	Outline of spores sharp	No change	No change

From the above, it is clear that the ammoniacal solution of copper oxide does not dissolve the spore membrane of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis*.

2) Lugol solution and sulphuric acid. Small pieces of filter paper were treated with Lugol solution, washed with distilled water, and were dried. They were mounted on slides in distilled water and in 50 per cent sulphuric acid respectively. Dried smears of spore emulsion of *Henneguya*

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salminicola, *Nosema apis* and *Nosema bombycis* were treated in a similar way. The results of observations are as follows:

	Soon after preparation
Cellulose: water and H_2SO_4	No coloration
Cellulose: Lugol	Deep brown
Cellulose: Lugol and H_2SO_4	Violet
Henn. salm : water and H_2SO_4	No coloration
Henn. salm.: Lugol	Slightly yellowish
Henn. salm.: Lugol and H_2SO_4	Slightly yellowish
Nosema apis and *bombycis*: water and H_2SO_4	No coloration
Nosema apis and *bombycis*: Lugol	Almost unstained
Nosema apis and *bombycis*: Lugol and H_2SO_4	Almost unstained

3) Zinc chloride-iodine-potassium iodide mixture. Fibers of a filter paper and dried smears of spores of *Henneguya salminicola*, *Nosema bombycis*, and *Nosema apis* were treated with the following mixture: Zincum chloratum pur. sicc. 20 gr., potassium iodide 6.5 gr., iodine 1.3 gr., and distilled water 10.5 cc. The results of observations are as follows:

	Soon after preparation	16 hours later
Cellulose with water	No staining	No staining
Cellulose with mixture	Violet blue	Violet blue
The spores with water	No staining	No staining
The spores with mixture	Slightly yellowish; iodinophilous vacuole of Henneguya brownish	No change

As will be seen from the above experiments, none of the cellulose tests gives positive reaction. It may therefore be stated as was remarked by Thélohan (1895) that the spore membrane of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* is not of cellulose nature.

c) Tests for chitin:—For the control of chitin test, I have prepared chitin from the wings of honey bees.

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1) Alkalies and acids. Dried smears of spores of *Henneguya salminicola*, *Nosema bombycis*, and *Nosema apis*, were treated with potassium hydrate solution and mineral acids. The results are as follows:

	Chitin	Spore membrane of *Henn. salminicola*	Spore membrane of *Nosema apis and Nosema bombycis*
Boiling KOH (35%)	Insoluble	Insoluble	Insoluble
Boiling dilute HNO_3	Soluble	Spore becomes more or less swollen; contents attacked	Spore becomes greatly swollen and hardly visible
Boiling conc. HNO^3	Soluble	Spore becomes larger; spore membrane of uniform thickness; less refractive	Spore becomes extremely enlarged; invisible.
Conc. HCl (room temp.)	Soluble	Slightly soluble; outline irregular	Spore enlarged and invisble
Boiling conc. HCl	Soluble	Attacked	Disintegrates rapidly
Dilute H_2SO_4	Insoluble	Insoluble	Insoluble
Conc. H_2SO_4 (room temp)	Soluble	Membrane becomes thinner; outline irregular; valves split; caudal filament broken	Greatly enlarged and invisible
Boiling conc. H_2SO_4	Soluble	Completely dissolved	Completely dissolved

2) Zinc chloride and Lugol solution. The spore emulsions of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* were mixed with potassium hydrate solution (35 per cent), and washed thoroughly by centrifugation. After being partly dried, 1 cc. of a mixture of 33⅓ per cent aqueous solution of zinc chloride (10 cc.) and of strong Lugol solution (5 drops) was added, and observed. The results follow

	Color reaction
Chitin	Dark brown
Henneguya salminicola	Spore membrane very slightly yellowish
Nosema bombycis and *Nosema apis*	Spore membrane unstained

3) Potassium iodide and sulphuric acid. Chitin and dried smears of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* were boiled at

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160°C with potassium hydrate solution (35 per cent) for thirty minutes, washed thoroughly with 90 per cent alcohol, and then with distilled water. They were then treated with a weak solution of potassium iodide which had been acidified with sulphuric acid, and were examined. The results are as follows:

	Color reaction
Chitin	Bluish violet
Henneguya salminicola	No visible staining of the membrane
Nosema bombycis and *Nosema apis*	No visible staining of the membrane

As will be seen from the above experiments, the staining reaction gives very ambiguous results. On the other hand, the effect of mineral acids upon the spore membrane seems to be decisive. The spore membrane of *Nosema bombycis* and *Nosema apis* behaves very much like chitin under the influence of mineral acids, while that of *Henneguya salminicola* is more or less different in this respect.

The Polar Filament

Thélohan (1890:207) expressed an opinion that the substance composing the wall of the polar capsule was identical with that composing the spore membrane, as both stained in the same way with safranin. This view probably led Minchin (1912:399) to state that "a polar capsule is a hollow, pearshaped body with a tough envelope, probably chitinoid in nature. . . . Coiled up within the capsule is a delicate filament, often of great length, probably of the same nature as the capsule, and continuous with it." Minchin does not give any evidence to support this view. Davis (1917:210) possibly referred to Minchin, although he did not make it clear, when he stated as follows: "Surrounding the capsule is a tough, refractive envelope, probably chitinous. . . . Coiled up within the capsule is a delicate filament, usually of comparatively great length, which is probably of the same material as the capsule."

Erdmann (1917:317), by studying the developing polar filament of *Chloromyxum leydigi*, came to the conclusion that the polar filament is composed of glycogen and plastin. She writes as follows: "Die vier Polkapseln füllen jetzt ihre Mutterzelle aus, die Plastinscheiben werden zu Plastinringen, die durch Glykogen verbunden sind. Der Polfaden ist

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entstanden. . . . Dagegen kann ich die Umwandlung des Chromatins and des Plasmas in eine stark färbbare Substanz, nach meinen Befunden, Glykogen, bestätigen." To support this view, Erdmann further remarked the difficulty in extruding the polar filament of spores of the species of the genus Chloromyxum as follows "Ausgestreckte Polfäden von Chloromyxumsporen sind kaum an Präparaten beobachtet. Thélohan (Taf. IX, Fig. 100c) bildet die Polfäden einer frischen Spore von *Chloromyxum quadratum* ab, Erdmann *Chloromyxum leydigi* mit kurzem Polfäden. Auerbach ist es nicht gelungen, bei *Chloromyxum dubium* sie zu zeigen. Lebzelter erwähnt sie nicht bei *Chloromyxum thymalli.* Durch eine von mir ausgeprobte Methode der Fixierung (100 Proz. Alkohol bis auf 40 Grad erhitzt) gelingt es leicht, die Polfäden zum Austreten zu bringen und zu fixieren. Glykogenfärbung nach Fixierung zeigt, dass der Polfaden aus Glykogen und einer plastinähnlichen Substanz zusammengesetzt ist (Taf. 14, Fig. 27)." I have, however, had no difficulty in causing the extrusion of the polar filament from the fresh spores of three out of five species of Chloromyxum which I have studied up to date. In *Chloromyxum misgurni* Kudo (Kudo 1916, Fig. 3e), *Chloromyxum fujitai* Kudo (drawings were omitted due to the lack of space) and *Chloromyxum trijugum* Kudo (Kudo 1919: Fig. 181), I have caused the filament extrusion. The other two species, *Chloromyxum catostomi* Kudo (Kudo 1919) and *Chloromyxum wardi* Kudo (Kudo 1919) were studied only in fixed specimens, and no attempt was made to cause the filament extrusion. Erdmann has probably studied a small number of spores.

Regarding the various methods which had been reported by several investigators as successful in extruding the polar filaments of various Cnidosporidian spores, I already summarized them in one of my papers (Kudo 1918). Of many methods which I have tried since that date, the following gave always the best results. For Myxosporidian spores, potassium hydrate solution or perhydrol will always bring out satisfactory results. This is especially true in the case of tissue infecting forms. When the spores are found in the gall bladder or urinary bladder, the best results are obtained by centrifuging the spore containing bile or urine followed by repeated washing with distilled water before the spores are subjected to the influence of the chemicals, although this is not of absolute necessity. Yet when the number of spores present in the bile or urine is very small, the treatment is favorable as the addition of potassium hydrate solution to the bile produces a large amount of precipitation which hinders the observation greatly. In most cases, no staining of the filament of Myxosporidian spores seems to be necessary, due to the favorable thickness and distinctness even in unstained state. For staining, Fontana's method of staining spirochoetes, or Giemsa's solution gives beautiful results. The

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latter has its advantage over the former in bringing out the differentiation of nucleus and sporoplasm beside the filament, although in some cases the filament does not take the stain for unknown reasons. For Microsporidia, mechanical pressure or perhydrol gives beautiful preparations of extruded filaments. As I did not describe the detail of the method used (Kudo 1913), I have recently described the exact technique for the application of mechanical pressure elsewhere (Kudo 1920).

Although Erdmann was apparently unaware of it, the filament extrusion under the effect of absolute alcohol had been described by Ohlmacher (1893) in the case of *Leptotheca ohlmacheri* (Gurley) Labbé. In the section preparations of kidneys of *Bufo lentiginosus* fixed with absolute alcohol, Ohlmacher saw a number of spores with extruded polar filaments. Ohlmacher was of the opinion that "it is, of course, evident that they (polar filaments) must have been thrown out from spores before the organisms were killed by the alcohol employed in fixing." According to my own observations on a large number of section preparations obtained from *Rana clamitans*, it is clear that in Ohlmacher's preparation, the fixation with absolute alcohol which caused vigorous shrinkage of the spore membrane and sporoplasm, was only responsible for the presence of spores with extruded polar filaments. It is certain that the absolute alcohol method of Erdmann is according to my comparative study on various methods far inferior in having incompleteness and irregularity in its action.

The results of my observations are as follows:

1) The effect of water upon the polar filament. To determine whether the extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Myxobolus mesentericus* and *Leptotheca ohlmacheri* are soluble in water or not, fresh spores were subjected to mechanical pressure. After removing the coverglasses, the smears were covered with distilled water, and were kept in a moist chamber. The examinations were done under a dark field microscope, and also in Fontana preparations. The results were similar in four species, which are as follows:

	Results of Observations
Control: soon after the application of mechanical pressure	Extruded polar filaments
One day in water	Polar filaments unchanged
Two days in water	Polar filaments unchanged
Four days in water	Polar filaments unchanged
Eight days in water	Polar filaments unchanged

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The experiments were repeated many times on other species than mentioned above, but always giving the same results. From these experiments it may be concluded that the polar filaments of the spores of Cnidosporidia mentioned above are insoluble in distilled water at room temperature.

2) The effect of filtered saliva upon the polar filaments. To determine whether the extruded polar filaments of spores of the species mentioned above are soluble in filtered saliva or not, fresh spores were pressed mechanically. A drop of filtered saliva was added to each smear, and the smears were kept in a moist chamber. The examinations were done as in the preceding experiments, and revealed the following results which were practically the same in the four species:

	Observations
Control: soon after the extrusion	Extruded polar filaments
10 minutes in saliva	Polar filaments unchanged
30 minutes in saliva	Polar filaments unchanged
1 hour in saliva	Polar filaments unchanged
3 hours in saliva	Polar filaments unchanged
16 hours in saliva	Polar filaments unchanged
32 hours in saliva	Polar filaments unchanged
3 days in saliva	Polar filaments unchanged
6 days in saliva	Polar filaments unchanged

From the experiments, it may be said that the extruded polar filaments of the spores of *Leptotheca ohlmacheri*, *Myxobolus mesentericus*, *Nosema bombycis* and *Nosema apis*, are insoluble in filtered saliva at room temperature.

3) Staining with Lugol solution. The extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Thelohania magna*, *Leptotheca ohlmacheri* and *Myxobolus mesentericus* stain uniformly as light yellowish as their spore membrane by Lugol solution, and do not take any deeper color.

4) Staining after Best's method. The extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Thelohania magna*, *Leptotheca*

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ohlmacheri and *Myxobolus mesentericus* remain unstained by Best's method.

5) Staining after Lubarsch's method. The extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Thelohania magna*, *Leptotheca ohlmacheri* and *Myxobolus mesentericus* stain uniformly slightly bluish-violet by Lubarsch's method. The polar capsules of the latter two species and the spore membranes frequently stain deep violet.

6) Staining by Löffler's method. The extruded polar filaments of spores of *Nosema bombycis* (Kudo, 1913), and of *Nosema apis*, *Thelohania magna*, *Leptotheca ohlmacheri* and *Chloromyxum trijugum* are stained deep violet by Löffler's method. The spore membrane is also stained in the same color.

7) Staining with Giemsa's stain. The extruded polar filament of *Nosema bombycis* (Kudo, 1916), and of *Nosema apis* and *Chloromyxum trijugum* (Kudo 1920, Fig. 181) have been stained in deep, dark red. The polar capsules of the latter species and the spore membrane also frequently stain the same color.

8) Staining with Fontana's mixture for staining spirochoetes. The extruded polar filaments of *Nosema bombycis*, *Nosema apis*, *Thelohania magna*, *Thelohania illinoisensis*, *Leptotheca ohlmacheri*, *Chloromyxum trijugum* stain in from yellowish to dark brown color by Fontana's method. The spore membrane takes the stain in the similar manner.

From these experiments, it is clear that the polar filaments of spores of various species of Cnidosporidia, which have been listed in the above, are not composed of glycogen as was thought by Erdmann in the case of *Chloromyxum leydigi*. The only means which led Erdmann to the already quoted conclusion regarding the chemical nature of the polar filament is the results of Lubarsch's staining. My experiments have shown clearly that while this staining brings out more or less bluish stained filaments, other tests for its glycogenous nature proved to be negative. The staining effect of Löffler's method is similar to that on the flagella of *Bacillus typhosus*, and that of Fontana's method is exactly the same as that on various spirochoetes.

As to its true nature, I am, however, still unable to determine. It has been noted by many investigators in numerous species of Myxosporidia, and by myself in *Leptotheca ohlmacheri* and *Thelohania magna* that the nucleus for the polar capsule becomes nebulous or diffused during the formation of the polar filament. The chromatic substance of the nucleus breaks up into numerous small granules and a large part of it unites with a peculiar substance or substances which become differentiated in the capsulogenous cell, first in a retort shape and then in rounded form. The polar filament is apparently formed from this mixture.

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The Iodinophilous Vacuole

In the mature spores of Myxosporidia belonging to the family Myxobolidae, there exists regularly a more or less conspicuous rounded space which is generally known as an iodinophilous vacuole because of its behavior toward iodine.

Müller (1841) seems to be the first to notice this peculiar structure and figured vacuoles in his drawings. Bütschli (1881:636) observed the vacuole in the spore of *Myxobolus mülleri* which had previously been seen by Müller, and designated it as a nucleus. He described the structure as follows: "Von besonderem Interesse erscheint das unzweifelhafte Vorhandensein eines Zellkerns in der plasmatischen Inhaltsmasse der Sporen. Häufig ist dieser Kern schon in frischem Zustand ohne Weiteres als kreisförmiger bis ovaler, heller Fleck recht deutlich sichtbar (Fig. 1n). Besser tritt er jedoch nach Behandlung mit verdünnter Essigsäure oder Jodtinktur hervor und zeigt dann eine dunkle, etwas granulirt erscheinende Hülle (Fig. 2n) und eine Anzahl ziemlich blasser Granula, welche durch den Inhalt zerstreut sind. Leider setzten sich dem Versuch, den Kern zu färben, sehr energische Hindernisse entgegen, da das Färbungsmittel nicht in die Sporenschale eindringt; jedoch kann dieser Umstand nicht gegen Kernnatur des fraglichen Gebildes angeführt werden, da auch das Plasma der Färbung widerstand. Dennoch beobachtete ich einige Fälle deutlicher Kernfärbung bei Anwendung von Alaunkarmin."

That Bütschli's view was not correct was shown by Thélohan (1899: 919–920) who studied the structure more closely with the following statements: "Si, en effet, on traite ces spores par différentes réactifs, on acquiert bientôt la certitude que la tache claire observée par M. Bütschli, et décrite et figurée par lui comme un noyau, est en réalité une formation d'ordre tout différent. Peu visible à l'état frais, à-cause de la transparence du protoplasma, elle apparaît plus nettement par l'action de l'alcool, des acides acétique, azotique, osmique ou du nitrate d'argent à 2 pour 100. On la voit alors entourée par le reste de la masse plasmique, qui, coagulée sous l'influence de ces liquides, se distingue par son aspect finement granuleux et sa moindre réfringence. Elle a tous les caractères d'une vacuole creusée au sein de cette mass et remplie d'une substance particulière, remarquable par sa résistance aux réactifs colorants caractéristiques de la substance nucléaire. Seul l'iode se-fixe sur elle, et, tandis que, sous son influence, le reste de la spore prend une coloration d'un jaune pâle, on voit cette vésicule devenir d'un rouge brunâtre qui rappelle absolument la teinte que prend la matière glycogène par l'action de ce réactif. Comme celle-ci, cette substance est insoluble dans l'alcool et grade sa réaction vis-à-vis de l'iode dans les spores conservées dans ce liquide. Comme elle encore, elle est soluble dans les alcalis. Les acides la modifient, et après leur action elle ne se colore plus. Toutefois, je n'ai pu, dans ces circonstances,

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obtenir la réduction de la liqueur cupro-potassique." Gurley (1894:209) is in entire accord with the observations of Thélohan quoted above.

Keysselitz (1908:264) is the only other investigator who studied rather closely the structure under consideration. He remarks as follows: "Die Vacuole hat eine rundliche Form; ihre Grösse ist nicht ganz konstant. An der lebenfrischen Spore kann man sie nicht oder kaum bemerken. Nach Behandlung mit Argentum nitricum, Alkohol, Osmiumsaure (Vgl. Thélohan) Aqua destillata, gewöhnlichem Wasser (bei einzelner Sporen) beim Erhitzen sowie beim Antrocknen tritt sie deutlicher als heller Bezirk hervor. Sie ist gegen das umgebende Plasma nicht durch eine deutliche Membran abgesetzt. Beim Zusatz von wässeriger oder alkoholischer Jodlösung, färbt sich ihr Inhalt mahoganibraun, eine Reaktion, die für die Sporen der Myxobolen specifisch zu sein scheint. Er erscheint dann zuweilen fast homogen, häufiger bemerkt man verschwommene dunklere und hellere Flecke verschiedener Grösse und Form. Der Inhalt scheint mir eine zähflüssige Substanz zu sein, die in der Zelle gleichsam suspendiert ist. In konservierten, mit Farbstoffen behandelten Sporen tingiert sich die Vacuole nicht. Sie imponiert als heller Fleck in der Copula. Durch das Jod wird in der Regel auch in den zwischen den Polkapseln befindlichen Räume ein kleiner nicht scharf umgrenzter Bezirk mahoganibraun gefärbt." Auerbach (1910:16) simply states that "die Vacuole färbt sich bei Zusatz von Jodtinktur braun."

To summarize the views advanced by previous authors, the so-called iodinophilous vacuole is stained with iodine mixtures, and therefore is of glycogenous nature.

My observations gave the following results.

1) The effect of distilled water upon the vacuole. In order to determine whether the vacuole is affected by disti led water in partly exposed conditions or not, fresh spores of *Myxobolus mesentericus* and *Henneguya mictospora* were crushed under the cover glass, and were kept in distilled water. After six hours, the smears were treated with Lugol solution, which gave the following results on examination:

	Observations
Control spores without being pressed	Typical coloration of the vacuole
Spores crushed	No visible staining of vacuole-like structure

The experiments show that the contents of the vacuole disappear when placed in contact with distilled water.

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2) Treatment with Lugol solution. Smears and section preparations of *Henneguya salminicola*, *Myxobolus aureatus*, *Myxobolus discrepans* and *Henneguya mictospora* were treated with Lugol solution. The vacuoles stained in brownish orange which on warming disappeared.

3) Staining by Lubarsch's method. Section preparations of *Henneguya salminicola* were stained in Lubarsch's mixture, the iodinophilous vacuole, the polar capsules as well as spore membrane were stained in deep bluish violet.

4) Staining by Best's method. Section preparations of *Henneguya salminicola* were stained by Best's method. The vacuole took a faint pink color.

5) Staining with Delafield's haematoxylin and Lugol solution. Section preparations and smears of *Henneguya salminicola* and *Myxobolus mesentericus* were first stained with Delafield's haematoxylin. After being washed thoroughly, they were mounted in gum and Lugol mixture. The iodinophilous vacuole stained in reddish brown.

From the experiments mentioned above, it is certain that the so-called iodinophilous vacuole of spores of the family Myxobolidae contains a substance similar to glycogen in characters.

In the section preparations of the cysts of *Henneguya salminicola*, one sees the appearance of the vacuole as the spore formation proceeds. The glycogenous substance in the sporoplasm remains inconspicuous while the spore is in the pansporoblast, although one can trace the gradual concentration of the substance in it. When the spore matures and separates itself from the other spore, the vacuole becomes sharply outlined. The vacuole reaches its maximum size when the spore is completely formed.

No particular body that corresponds to the iodinophilous vacuole was found in the spores of species belonging to families other than Myxobolidae, although I have tested several species repeatedly.

It is generally understood without any experimental evidence that the glycogen occuring in the spores of the family Myxobolidae is probably used for the future development of the sporoplasm. Then it is strange to notice the fact that the spores of other families which are essentially the same in habitat and in many other respects, do not contain the glycogen in such a conspicuous way as in this particular family. The majority of species belonging to the family Myxobolidae attack the tissue of the host, yet some species of the genera Chloromyxum, Myxidium and Sphaerospora and all the species of the family Myxosomatidae which do not show any iodinophilous vacuole in the spore at any stage of its development, inhabit also the tissue of the host. Therefore the occurrence of the iodinophilous vacuole does not seem to be correlated with the tissue infesting characters of the Myxosporidia, as was suggested by Gurley (1894).

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Summary

1) The spore membrane of *Nosema apis* and *Nosema bombycis*, taken as representatives of Microsporidia, is proved to be composed of a substance similar to chitin in its chemical reaction.

2) The spore membrane of *Henneguya salminicola*, taken as a representative of Myxosporidia, is proved to be composed of a substance, the chemical reactions of which are less similar to those of chitin compared with the microsporidian spore membrane.

3) The polar filaments of cnidosporidian spores are not composed of glycogen as was suggested by Erdmann. They are formed by the mixture of a part of the nucleus and a substance differentiated in the capsulogenous cell.

4) A review of the methods which cause the filament extrusion in Cnidosporidian spores is presented.

5) The so-called iodinophilous vacuole of the spores of the family Myxobolidae contains a substance, the chemical reactions of which are similar to those of glycogen.

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DEPARTMENT OF SUMMARIES
DEVOTED TO DIGESTS OF PROGRESS IN BIOLOGY

RECENT ADVANCES IN PARASITOLOGY[1]

By Ernest Carroll Faust

Parasitology has made extraordinary progress during the last decade. In writing on this topic ten years ago Ward (1910) states increasing interest in problems of medical zoology had made the mass of material at that time so vast that a review of it was difficult. Since then stupendous progress has been made.

These advances have been due in part to the more general recognition of the importance of this science and the relation of its development to human welfare and in part to the stimulus of the World War. While individual investigators have contributed a great wealth of valuable data to the science, the most outstanding discoveries have come as the result of the work of commissions and bureaus, undertaking fundamental problems of parasitology on a comprehensive scale. Such accomplished facts as the eradication of yellow fever and the minimization of malaria in the Panama Canal Zone, the Hookworm and Tuberculosis campaigns of the International Health Board, and results of the Bilharzia Mission in Egypt are outstanding monuments of progress during this decade.

In attacking the problems in hand the life history of the parasite has been frequently worked out. As in the solution of previous protozoan and helminth diseases, a knowledge of the life history has not only been valuable but in most cases the essential factor in the eradication of the evil. Such a knowledge has shown the most practicable way of breaking the vicious cycle.

As a result of the World War world problems have developed in parasitology from what were formerly matters of Oriental or tropical concern. Troops coming from countries subject to tropical diseases, returning home, have brought infections with them. Such is quite likely the case in such protozoan diseases as amebiasis which require no intermediate host for part of their life cycle. But, in addition, the added impetus which has resulted from the study of such infections as World War problems, has made it evident that the pre-war infection in England and America, for example, was much higher than had previously been believed.

The period has been marked by the development of new laboratories and intensive study of parasitic problems in new fields. In part this

[1] Contributions from the Department of Pathology, Peking Union Medical College, Peking, China.

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work has been done by investigators, who, native to the region, have gained distinction in such problems. In part it has been accomplished by commissions which have been sent into the country to make these researches. In part it has been brought about by the efforts of those, who, distant from the field, have diligently sought out a solution to the problems, brought to them by explorers.

The progress in parasitology has been stimulated and cooperation of investigators secured by the appearance of several new Journals devoted entirely or for a major part to parasitology. First must be mentioned the Tropical Disease Bulletin, London, 1913, a review of all the important literature on the subject of tropical parasitology and medicine. The establishment of the Journal of Parasitology (1914) in America affords opportunity for publications of investigations of a type midway between Parasitology of Cambridge and the Annals of the Liverpool School. More recently the Kitasato Archives of Experimental Medicine has entered the field, affording opportunity for workers in the Orient to publish near at home. Just recently a long-felt want has been filled by the appearance of the American Journal of Hygiene and the American Journal of Tropical Medicine.

Continuing the task of placing in the hands of investigators a dependable and indispensable index of Medical and Veterinary Zoology, Stiles and Hassall (1912) have published their Index-Catalogue on Cestoda and Cestodaria. Its value over the Trematode Catalogue of the series consists in the more thoro analysis of specific and sub-specific citations with the page reference for each and in the fewer number of errors which inevitably creep into a work of such scope. The long-awaited companion volume on Roundworms has recently been issued (1920) and meets the expectations of the most critical reviewer. Along this line one cannot commend too highly the synopses of important papers relating to medical parasitology appearing in the Tropical Disease Bulletin. It is to be regretted, however, that the reviewers of this Bulletin have not seen fit to include certain other reviews which, altho technically non-medical, are fundamentally related to medical problems.

New species and new systematology in helminths are brought together in a most workable digest in the chapters on Platyhelminthes and Nemathelminthes in Ward and Whipple's Fresh-Water Biology (1918). Progress in American helminthology is shown in the fact that many of these species are described for the first time and in the introduction of a considerable portion on cercariae to the subchapter on trematodes. The data are made especially valuable by their relation to one another in the form of a key, and are made the more workable by ample illustrations.

The most comprehensive treatise on the subject of human parasitology which has appeared within the decade is Fantham, Stephens, and Theo-

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bald's "Animal Parasites of Man" (1916), a book which has no equal in point of completeness and in up-to-the-minute information on human entozoa. The writing of a brief review of progress in this field gives an insight into the monumental character of this book. While strides have been made in the science ever since the publication of "Animal Parasites" it remains the reliable compendium and guide to the researcher or practitioner encountering entozoic ailments. In their Manual of Tropical Medicine (1919) Castellani and Chalmers have not only contributed greatly to the knowledge of tropical protozoa, helminths and arthropods from data largely drawn from their own wealth of experience in the Tropics, but they have likewise secured the permanent cooperation of the practitioner in problems of parasitology by presentng the clinical and pathological pictures of these parasitic infections. The manual stands as a lasting memorial to the junior author, who gave his life for the work.

Perusal of the literature of parasitology of the period which is covered in this review reveals a vast wealth of investigation, the major part of which falls within the group of the protozoa. Workers on protozoa have been many and a considerable share of their contributions significant. Certain problems like amebiasis have been studied in new fields. In other cases the life history has been elucidated. In many cases, however, mere symptomatology and diagnosis have been set down, where the lack of new data hardly warrants more than a statement of the case.

Foremost among workers in protozoology are those of the English Schools, comprising Stephens, Fantham, Nuttall, Yorke, Macfie, Wenyon and Porter. With these investigators life histories have played an important rôle. With them, too, detailed descriptions of morphological features have not been neglected. One is most convinced of the thoroness of the work of the Liverpool School in reviewing the elaborate and most detailed methods which have been followed in the experimental treatment of malaria.

Work on the Continent of Europe of a high character has been done by Laveran, Leger, França, Negri and Galli-Valerio. In the Americas Craig, Kofoid, Darling, Hadley, Chagas and Magalhaes have made noteworthy contributions, while Cleland's solution of dengue in Australia and Miyajima's studies on the tsutsugamuchi deserve the highest praise.

In the words of Wenyon (1915) our knowledge of trypanosomiasis and malaria has reached something like full fruition. Hardly so much can be said of the majority of protozoon infections, partly because the circumstances have not been favorable, partly because the investigations have been side tracked.

In 1911 Novy touched upon the progress that had been made in our knowledge of protozoan infections and their treatment. The life history of *Trypanosoma brucei* had just been demonstrated (1909) and remained

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one of the outstanding discoveries of the decade. Little was known of the spirochaetes and their pathogenicity aside from the studies on treponema. The life history of the malarial plasmodium had been well authenticated, but other hematozoon forms were little known. Since then many groups have been carefully studied. Nuttall (1913) has found the life cycle of Babesia in dogs, horses and cattle to pass thru certain ticks as intermediate hosts and has discovered curative salts for these infections. Stephens (1914) describes a new tertian malarial parasite, *Plasmodium tenue*, from the Central Provinces, India. Yakimoff (1917) contributes to the knowledge of Piroplasma, Theileria, Nuttallia and Anaplasma infections of domestic and semi-domesticated animals of Russian Turkestan. Fantham (1910) and Hadley (1911) have given a clear morphological analysis of *Eimeria avium*.

Again, the studies of Ross and Thomson (1916) on Egyptian sand amebae show the necessity of preventing contamination of dry sand with fecal matter.

Wenyon and O'Conner (1917) have helped to solve the practical treatment of the protozoan infections of man in Egypt. They have been able to standardize treatment of amoebiasis. Three new human Parasites, *Waskia intestinalis* and *Tricercomonas intestinalis* and *Entamoeba nana* have been found in these studies.

Craig (1917) has established a basis for classification of amebae parasitic in man which allows one to profit from his numerous investigations in this field. He recognizes as valid species, *Craigia hominis* and *C. migrans*, *Endamoeba coli*, *E. histolytica* and *E. gingivalis*, and *Vahlkampfia lobospinosa*. A more conservative standard is presented by Dobell in his monograph on the amoebae (1919), a treatise which for its thoroness commands the attention and admiration both of the theoretical and the practical parasitologist.

Von Prowazek (1913) has published an important paper on *Balantidium coli*. He has carefully reviewed the geographical distribution of the species, described its histology in minute detail and methods of propagation, and has worked out its pathogenicity.

Work of the character of Fantham and Porter's (1914) contribution to the life-history of *Nosema bombi* has been of increasing importance in elucidating the general knowledge of protozoan life cycles and thus contributing indirectly to a knowledge of related human forms where experimental infections are obviously less possible. In reviewing the work on protozoa one is struck by the mass of such work of an excellent character of which lack of space unfortunately does not even permit mention.

Watson's monograph (1916) on Gregarines constitutes a well organized synopsis of new and described species of the group, many species of which had previously been investigated only piecemeal.

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Dobell (1918) has contributed a valuable memoir in his study of human coccidia. Following up the work of Wenyon (1915) he has described three definitely known species infecting man (*Isospora hominis* Riv. 1878, *Eimeria wenyoni* n. sp. and *E. oxyspora* n. sp.,), in addition to throwing doubt on the identity of a third Emeria species as that of the rabbit (*E. stiedae*).

Moroff (1915), after a searching investigation, places the sarcosporidia close to the gregarines and coccidian forms in the subclass Telosporidia, along with the Haemosporidia.

Wolback's work on the Rocky Mountain spotted fever (1918) has shown that the causal agent of the disease is a minute parasite, present in the blood of infected mammals and in ticks which are capable of transmitting the disease.

Kudo's monograph on the Myxosporidia (1920) is memorable not only as a collation of the work of earlier investigators, but as a survey of the large number of myxosporidian forms studied by Kudo himself.

The work of Poche (1913) on the System of Protozoa is a comprehensive treatment of nomenclature of the group. It is notable for the large number of new orders, suborders and families proposed, many of which are readjustments of rank justified by the increase in number of the group. With the wealth of knowledge of morphology and life histories of the Protozoa careful systematic readjustments of this type are increasingly necessary.

Work on the helminths has been continued by many of the investigators who have already established a name for themselves among parasitologists. In Europe Odhner has contributed further studies to his work on phylogeny and systematology, among the most interesting of which are those on Schistosome and Holostome groups. Goldschmidt has extended his investigations on cytology most successfully. Kossack has monographed the monostomes, while Monticelli and Lühe have contributed much to the knowledge of trematodes. Fuhrmann, Leon and von Ratz have studied the cestodes while Railliet and Henry and Seurat have made notable contributions to the nematodes. The most brilliant work of the younger helminthologists in Europe is undoubtedly that by Leiper.

In America such studies have been continued by Ward, Ransom and Young on Cestodes, by Ward on Trematodes, and by Ward and Ransom on nematodes. In addition there has arisen in the United States a considerable group of younger investigators, of whom La Rue, Cort, Boeck and Van Cleave deserve prominent mention.

In Japan Katsurada, Fujinami and Goto have produced work of high merit. Yoshida, Okanama, Kobayashi and Miyairi have added much to life-history problems.

The contributions on Australian helminths count among their number the investigations of Nicoll, Cleland, S. J. Johnston, T. H. Johnston,

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Breinl and Sweet. This summary of important contributors to the science would not be complete without mention of Ssinitzin, Skrjabin and Yakimoff for Russia and Southwell for India.

Ward (1917) has emphasized the necessity of rearranging forms "so as to express better their correct relationships in the light of more perfect knowledge of their structure." But he adds the essential corollary that it has been his fixed principle never to make any change until he was personally familiar with the form discussed or had acquired such acquaintance with its structure as to know that some change was inevitable and that the proposed modification was defensible on morphological grounds. On this basis he has made fundamental but conservative changes in trematode and acanthocephalan groups and has established order in the nematode group where previously taxonomy was confined most usually to mere descriptions of new species.

The period has experienced an advance in helminthology from an almost purely zoological science to one ministering to the needs of comparative bionomics and medicine.

The outstanding morphological and systematic contribution to our knowledge of the Cestoda during recent years is La Rue's Monograph on the Family Proteocephalidae (1914). Provided with a wealth of American material, supplemented by more than on ordinary amount of types of the group described by European and other workers, La Rue has been enabled to mold the material into a comprehensive and practically exhaustive treatise. His descriptions and drawings are detailed, yet clear, his types are well defined and the amount of material collected, the amount used in study and the location of each specimen in the collection are minutely set down. Added to this are valuable synoptic tables and a workable natural key to the group. The contribution as a whole is such as to place the author immediately in the rank of the foremost helminthologists.

Recently Cooper (1919) has monographed another group of cestoda from fishes which contributes to our knowledge in that group.

Among other contributions on cestode anatomy and phylogeny the work of Douthitt (1915) on Anoplocephalidae is worthy of mention. Because of the care which this investigator used in working over his material and the gradual way in which he built up a natural classification of the group the monograph will serve as a lasting memoir to his efforts.

Ransom (1913) has made possible the statement that *Cysticercus ovis* is the intermediate stage of a dog tapeworm, *Taenia ovis* (Cobbold) Ransom and in working out the life-history of this cestode experimentally has solved a problem of long standing. This species in the bladder-worm stage has thus been proved to be distinct from *Cysticercus cellulosae* and the adult from *Taenia tenella*, *T. solium*, *T. hydatigena*, and *T. marginata*. Treatment of dogs for the tapeworm is found not only to eradicate this per-

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plexing economic problem of sheep measles but rids them of other worms of equally serious pathogenicity.

Beddard (1911–1914) has contributed studies from time to time, making known to science a large number of cestodes parasitic in animals in the Zoological Society Gardens (London). Likewise Skrjabin (1914) has contributed to our knowledge of the Cestoda of birds of Russian Turkestan. Fuhrmann (1918) in a detailed survey of the avian cestodes from New Caledonia and Loyalty Isle adds materially to the knowledge of the families Tetrabothriidae, Anoplocephalidae, Davaineidae, Dilepididae, Hymenolepidae, Acoleidae and Amabiliidae.

Thus the comparative work on cestodes has been greatly advanced.

The striking advances in our knowledge of the trematodes have come as life-history problems. Members of the medical profession have been especially sympathetic to this work because it was concerned with flukes most of which affected man. It is particularly noteworthy that all of these without exception have borne out the principle established for *Fasciola hepatica*, that the miracidium penetrates a mollusk, and from the mollusk the cercaria emerges which reaches its definitive host (immediately or intermediately according to the group to which the species belongs) and there becomes mature. Faust (1918) following Ssinitzin's work (1911) has found the sporocyst and the redia stages to be true parthenitae.

A problem which Looss had repeatedly attempted to solve in Egypt and on which Katsurada and Fujinami have contributed much in Japan was the schistosome life history. Credit for the first solution of the life cycle is due to Miyairi and Suzuki (1914) in Japan and later to Leiper and Atkinson for Japanese species and Leiper (1915) for the two Egyptian species. A clear understanding that the miracidium enters a gasteropod and that by change of cycle the cercaria emerges from the snail and directly infects man thru the skin or the mouth has made possible methods for preventing the disease. It has also made possible a clear restatement of the thesis that "The larval metamorphosis of all digenetic trematodes occurs without known exception in the bodies of molluscs belonging to the classes Gasteropods and Lamellibranchia." Leiper (1918) has shown that when once infected the patient is practically incurable. He has found from his Egyptian Researches that

"(1) Transient collections of water are quite safe after recent contamination.

"(2) All permanent collections of water, such as the Nile, canals, marshes and birkets, are potentially dangerous, depending upon the presence of the essential intermediary host.

"(3) The removal of infected persons from a given area would have no effect, at least for some months, in reducing the liability to infection, as the intermediate hosts discharge infective agents for a prolonged period.

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"(4) Infected troops can not reinfect themselves or spread the disease directly to others. They could only carry the disease to other parts of the world where a local mollusc could efficiently act as a carrier.

"(5) Infection actually takes place both by the mouth and through the skin.

"(6) Infection in towns is acquired from unfiltered water which is still supplied, ever in Cairo, in addition to filtered water, and is delivered by a separate system of pipes.

"(7) Eradication can be effected without the cooperation of infected individuals by destroying the molluscan intermediaries."

Nakagawa (1916) has unravelled the life cycle of *Paragonimus Westermani*, showing that the cercaria is developed in Melania species and the encysted larva in Potamon. These infected crabs when fed to puppies gave rise to typical pulmonary paragonimiasis. Moreover the route of infection has been found to be from the intestinal wall in the vicinity of the jejunum, thru the abdominal cavity, thru the diaphragm and pleural lining, where it bores thru the lung tissue and encysts. Yoshida, working on the same problem entirely independently in Japan, was able to substantiate Nakagawa's results. Kobayashi's work on this fluke in Korea (1918) has hardly as convincing data as those of his colleagues. On the other hand the latter investigator (1915) has clearly shown experimentally that the encysted larva found in several species of Japanese fresh-water fish develops into the human fluke, *Clonorchis sinensis*. Thus far, however, he has not worked out the cercarial phase of the life-history of this worm. Nakagawa (1921) has just published his experimental work on *Fasciolopsis buski*, which he finds to infect the hog as the encysted post-cercarial distomule.

Recently interest in larval trematodes has been revived and the field for study of this group in America has been studied by Cort and Faust, who have shown that specific marks of discrimination in cercariae are important even tho they are minute. These investigators have added data on the larvae which should facilitate life history studies on flukes. Among these studies are those on flame-cell constancy and homology, including the use of this system as a basis for systematic relations. Of importance both to pure science and to medical parasitology, Cort's monograph (1919) on the cercaria of the Japanese blood fluke sets a record for careful study and detail in this group. Furthermore, Cort's study on the stages of development of the schistosome in the definitive host (1921) makes a valuable addition to the ontogeny of the fluke.

One can not overlook the researches of Ssinitzin (1911) in this field. This investigator has not only presented data on many interesting and unique larval flukes, but has presented theories of their phylogenetic relations which are at least extremely suggestive and stimulating.

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A morphological paper which has done much to show the necessity of exactness in differentiation of closely related species is that of Ward and Hirsch (1915) on the species of Paragonimus. These authors have found the type, size and group relationships of the spines to be distinctly diagnostic, and this fact, coupled with the importance of one of these species to medical science in the Orient makes the work especially significant.

Comparatively few investigators have made important studies during the past decade on the morphology and systematology of parasitic nematodes. Railliet and Henry in France and Ward and Magath in America have published researches which constitute marked exceptions to this lack of such investigation in this group. The first significant analysis of the parasitic Nematodes in America is embodied in Ward's chapter on these worms in Ward and Whipple's Fresh-Water Biology (1918).

A most important contribution to the morphology of the nematode is embodied in Magath's monograph of *Camallanus americanus* (1919). This thesis constitutes the most significant work on a single nematode species since the researches of Looss on *Ancylostoma duodenale.* The writer describes in detail the organs and systems of the worm and arrives at the conclusion that formulae for measurement are not dependable but that where doubt arises in systematalogy there remains only the accurate morphological description of every organ and part of the form in question.

The most widespread campaign ever undertaken by governmental or private interest for the eradication of a particular disease is that which was undertaken by the Rockefeller Foundation for the banishment of hookworm from the earth. In 1909 the Rockefeller Sanitary Commission was created to combat the hookworm in the United States. The findings of this Commission of the prevalence of the worm, the "arrest of physical, mental and moral growth, great loss of life, and noticeable decrease in economic efficiency," together with the success which attended treatment of hookworm infection, led to the establishment in 1913 of the International Health Commission (afterwards known as the International Health Board) with the purpose in view of "extending to other countries the work of eradicating hookworm disease as opportunity should offer" and, so far as practicable, to follow up the treatment and cure of this disease with the establishment of agencies for the promotion of public sanitation and the spread of the knowledge of scientific medicine.

Forthwith this commission proceeded to determine 1) the geographic distribution and the approximate degree of infection, 2) to examine microscopically the cases and cure those infected, and 3) to establish sanitary conditions which would prevent soil-pollution.

At the close of 1918 the Board had solely or cooperatively attacked the problem in the Southern United States, Central Mexico, Cuba, Porto Rico, Jamaica, a considerable share of South America, Egypt, Ceylon,

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Siam, The Malay, South China, New Guinea, Papua, Java, Guam, and Queensland, Australia, and new work was under way in the Madras Presidency, India.

While the intensive method of microscopic examination and treatment of patients within a limited area was utilized, the more fundamental purpose of the campaign has been to develop an education propaganda for better sanitary conditions so that the sources of infection will be eliminated.

One of the fundamental life-history problems which has engaged the attention of investigators in several geographically different centers is that of Ascaris. Captain Stewart of the Indian Medical Service (1917, 1918) has shown that *Ascaris lumbricoides*, and *A. mystax* can be developed to a certain larval stage in the mouse and rat.

Ransom and Foster (1917, 1919, 1920) have been able to produce individuals more nearly mature in the sheep and goat. The latter writers have shown, however, that these stages of development in animals other than the hog and man do not necessarily imply that the mouse, or rat, sheep or goat serve as intermediate hosts for these parasites. Yoshida (1919), working on guinea-pigs, was able to trace the life history as follows: "The ascarid larvae escape from the egg shell in the intestine of the host and proceed to the abdominal cavity by boring through the wall of intestine. Thence they pierce the diaphragm to enter the pleural cavity, finally penetrating into the lungs from their surface. . . . Furthermore, the lungs are the only necessary and important organ to be passed by the larvae in the course of their development. . . . (They) continue their development and migrate to the mouth cavity through the trachea, again passing down the alimentary canal to the intestine of the host.

Work on the Acanthocephala has been relatively meager. Lühe's digest of the group (1911) has given a basis for Continental investigations while Van Cleave's numerous studies on American species constitute marked progress in methods and thoroness of investigation.

Almost the entire amount of our knowledge of insects in the rôle of carrier and intermediate hosts of parasitic disease has come within the last few years. It is within this period that the life histories of the trypanosome, the piroplasmas and the spirochaetes have been shown to develop in specific flies, fleas, bugs or lice as the case may be. Moreover, certain tapeworms, especially those of poultry, have been just recently shown to develop as larvae within insect hosts. Finally there is further proof of the importance in the spread of parasitic disease when the insect acts merely as a vector. For these reasons important campaigns against these several insects have been carried on by private forces and by government agencies, foremost of which is the all but complete eradication from the Western Hemisphere of yellow fever by controlling the mosquito transmitting the

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disease, with plans under way for a campaign on this insect in the remaining locus of infection.

Thus a considerable share of the problems which confronted parasitology at the beginning of the decade have been carried to completion while others are being gradually sifted out. In their place, however, have come still others which require the greater skill and the wider point of view for their full solution. All of these signs of progress in parasitology indicate that this science is rapidly coming to assume the place which it deserves as the companion of bacteriology and gross pathology.

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IMPORTANT LITERATURE ON PARASITOLOGY

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

A METHOD FOR ORIENTING AND MOUNTING MICROSCOPICAL OBJECTS IN GLYCERINE

BY

CHARLES BULLARD

The object of this paper is to describe a method of mounting desmids or similar microscopical objects in glycerine, so that they may not only be drawn in different positions with the camera lucida, but may form the basis of an herbarium of mounts which may be regarded as permanent, since the oldest preparations made by this method, now about twenty-five years of age, show no signs of deterioration. The account herewith presented is offered for the purpose of enabling others to utilize it, as well as to answer certain inquiries that have been made in regard to the subject. In its details the procedure presents no novel features. Its principles are those used in the laboratory of Professor Thaxter for mounting the lower fungi and other of the more delicate Thallophytes in glycerine, or glycerine and eosin, and sealing with King's Cement.[1] This method is especially well adapted for mounting desmids for study from the point of view of the systematist; since, in the vast majority of species only the well developed empty cells and semi-cells are useful for this purpose. Those species of Cosmarium or of Spirotaenia, for example, the cell-contents of which have to be studied, require more exact fixation methods, with which this paper is not concerned.

In mounting a sufficiently large object, no "finding ring" is needed. But for marking the position of a small or large series of minute objects, it is often essential. A ring of Brunswick Black has been found most serviceable for this purpose, and should be prepared months in advance, so as to become well seasoned. It may be placed centrally on the slide by means of the turntable, and need not be larger than the field of the low power.

THE MATERIALS AND THE METHOD OF THEIR USE

Since the procedure here described involves the use of a weak glycerine jelly as a means of orientation, it is necessary, in order to avoid the diffi-

[1] King's Cement was an invention of the Rev. J. D. King of Cottage City, Massachusetts who did not publish the formula or method of preparation. There is an antiquated recipe published on page 235 in Rev. A. B. Hervey's translation of Behren's "Guide to the Microscope in Botany" (S. E. Cassino Co., Boston 1885). Dr. Hervey assures me that it is genuine, as he received it directly from King. If the more modern form of this cement cannot be obtained from a dealer, it can be bought of its present maker, Professor R. E. Schuh, Howard University, Washington, D. C.

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culties inevitably associated with the process in warm weather, to perform the manipulations indicated in the cooler part of the year. After the objects are oriented, the weak jelly may be satisfactorily set by placing the slide-box containing the preparations outside a window where it will be chilled. The drawing can be done in the warm part of the year, when the light is also best. This weak jelly is made from any good clear glycerine jelly, such as that prepared by Kaiser's formula. A few drops of melted jelly in a small vial is reduced by the addition of boiled or distilled water, until the mixture will just set at the ordinary temperature of the room. It should be perfectly limpid. The cork may be furnished with a dropper, by pushing into its lower end a piece of platinum wire of such length that a small loop at the lower end nearly touches the bottom of the vial. The vial should be kept well corked.

Objects may be lifted, transferred and oriented by means of a fine needle such as "No. 12 Sharps." The needle is pushed eye first into the end of a large match of straight grained wood, until a quarter of an inch, or less, of the pointed end, which is thus as rigid as possible, remains projectting. A smaller instrument may be made by the addition of a proper bristle, for which purpose a carefully selected whisker of a cat or dog answers admirably; since it combines stiffness and elasticity with an extremely delicate point. This bristle should be cemented to the mounted needle, and bound in place by means of a long human hair, or a fine waxed silk thread, in such a position that the point of the bristle projects slightly. It is important in order to obtain the necessary rigidity that the free portion of the bristle should be as short as possible consistently with convenient use.

The half inch circular cover glass is best adapted for general use, and in mounts of this nature, it is necessary to employ a shallow cell. This may be readily made by supporting the cover at one side by either a somewhat compressible, or an entirely rigid support. The latter results n a better mount mechanically, the former is easier to work with. The more flexible support, consists in fibers of blue blotting paper completely picked and teased out and then felted together again into a ball by means of the forceps. This material has the advantage of compressibility, which permits one to vary a little the amount of glycerine used for the mounting medium. It has a certain disadvantage, however, from the fact that, as it is not a rigid support, care must be taken that a mass of sufficient size is used, to prevent the cover from touching the object, after the cement has dried and contracted. A rigid cover glass support may be made by selecting a very thin cover glass and placing it in an elongated folded paper. If this is pressed against the edge of the table and drawn back and forth, the glass within will be finely comminuted. A minute fragment of this may then be

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used to support the cover. One must learn by experience the approximate amount of glycerine needed to fill the space beneath the cover.

The cement may be kept in an ounce, or half-ounce, wide mouthed bottle. Into the bottom of the cork a match, bearing the ringing brush is inserted; the point of the brush nearly touching the bottom of the bottle. The ringing brush itself should be the smallest obtainable with not many but rather long hairs. A match may be pushed into the quill and firmly bound with silk, and the whole fitted to the cork as mentioned.

For a mounting medium it is best to use only chemically pure glycerine, filtered if necessary. It may be kept in a vial to the cork of which is fitted a platinum loop, or a properly selected mourning pin, by means of which the glycerine may be conveniently applied.

Manipulations

The details of the procedure for mounting large and small objects are the same, but the smaller species are obviously the more difficult. A slide is prepared for the reception of the objects by placing a minute smear, or streak of the weak jelly, within the ring previously prepared; or if desirable a series of minute drops may be used. It is convenient to employ an ordinary dissecting stand, with a x12 aplanatic triplet, on which the slide thus prepared is left, with the focus and light exactly regulated. The material to be mounted having been spread out in glycerine on a slide, the particular individual desired is selected under the compound microscope, and pushed about with the needle beyond the edge of the glycerine until freed from glycerine and all extraneous matter. In this condition it will readily stick to the needle, and can thus be lifted from the slide, transferred to the surface of the weak jelly, and there left until all the individuals desired for this mount have been transferred. The weak jelly should then be liquefied by breathing gently upon it, and the slide at once placed under the low power of the compound microscope. It will then be found that, with a little practice, the objects can be easily and systematically arranged with the needle. They may be set up in lines, or curves, in whatever order may prove most convenient. Should the jelly harden too rapidly, it may again be liquefied as above described. In a short time, one acquires skill in setting up objects, such as desmid semi-cells, in different positions under the compound microscope, without disturbing those already in position. For drawing care should be taken so to set up a symmetrical object that its vertical axis coincides with the optical axis, or nearly so. The horizontal axes may be pointed in any direction, by merely revolving the slide on the stage. After placing and orienting the specimens, the slide may be put away upside down in a slide box, until two or three more are brought to the same state of preparation. In this con-

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dition they may be left outside a window all night in order to harden the jelly.

When the preparation is ready to mount and seal, the support of blotting paper, or cover chip, already mentioned, should be placed near the edge where it will be just included by the cover as it is lowered. A clean drop of glycerine should then be placed on the object, sufficient to fill the cell as exactly as possible. If blotter shreds are used, and the glycerine does not quite fill up the cell after the cover is placed in position, cautious pressure over them with the point of the needle will spread the glycerine, and fill the cavity completely. It may then be carefully sealed. If more glycerine must be added to fill a cell, a small drop should be placed at a short distance from the edge of the cover, and a narrow streak of it drawn with the needle to the edge of the cover; so that a little will flow beneath it. After repeating this process with the needle till the cell is completely filled, the surplus glycerine must be carefully wiped off.

The complete removal, before sealing, of this surplus is absolutely essential for the preparation of a permanent mount; since this is the only way to prevent subsequent leakage. A very little cement under the edge within the mount serves to make it stronger. In order to remove all trace of glycerine, it must be very carefully wiped off by means of an old, much washed handkerchief, folded over the end of the forefinger in such a manner as to form a point, which is moistened with alcohol. This can safely be pushed up till it touches the edge of the cover, and then repeatedly renewed and worked around it unti. all glycerine is removed. In case a still larger surplus of glycerine must be removed, it is convenient to use small strips of blotter folded Λ shape. One end is moistened with alcohol and pushed up against the edge of the cover. Several pieces about the cover edge absorbing simultaneously will gradually remove most of the excess, after which, the slide must be very carefully cleaned with the handkerchief, as before mentioned, and the mount at once sealed.

When there is only one specimen, and different aspects of it must be drawn, the preparation is demounted after the first figure is made, and before sealing the mount. The cover is lifted off, the glycerine drained away, the jelly again liquefied as before, and the object transferred to another prepared slide, remounted in a new position and drawn again. The last remount, showing the object in its most characteristic position, may be sealed.

To seal a perfectly cleaned mount, it may be held in the unsupported left hand, while with the right, a light ring of cement is applied, after resting the right hand on the left. With a full brush, a drop of cement is then started on the edge of the cover, the hands being held as before, and led around the circle by means of the brush in such a manner that the

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cement ring is partly on the cover and partly on the slide. The first ring thus helps to keep the second under better control. Additional rings may be applied on the turntable, when the sealing rings are hard. It has been found convenient to finish with two coats of Brunswick Black. This is soluble in turpentine, and offers more resistance to the solvent action of alcohol used to clean immersion oil from the cover.

In glycerine mounts of most objects, collapsed specimens regain their turgescence; and air bubbles, if present, disappear in a few days. Since these mounts are delicate, they must be handled with care, and always kept horizontal. Any necessary modifications of this method may be made in order to mount other microscopical objects needing orientation for camera drawing, and thus make it possible to obtain more accurate figures. But the fact that mounts thus sealed have shown no signs of leakage for so many years, indicates that the correct principles have been applied at this critical point, and should not be lightly changed.

Cambridge, Mass., April, 1921

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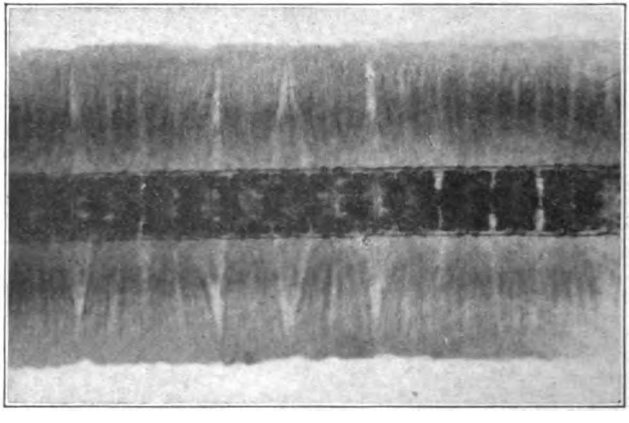
A METHOD OF DEMONSTRATING THE SHEATH STRUCTURE OF A DESMID

The structure of the cell wall in the Desmids is intimately concerned with the method of formation of the mucilaginous sheath, which in many members of the group is found to surround the cell. In the Saccodermae the wall is believed to be continuous thruout, having no pores communicating with the exterior. On the other hand, the Placcodermae in addition to other distinguishing characteristics frequently show pores connecting the protoplast with the surrounding medium.[1] In the Placcodermae it is considered that the mucus exudes thru the pores, and may accumulate outside the cell wall, so forming the sheath. It is not usually possible to observe directly evidence of this extrusion, but in the filamentous desmid *Hyalotheca dissiliensis* (Sm.) Bréb. the sheath shows under reduced illumination striae radiating from a zone around the ends of each cell.



HYALOTHECA DISSILIENSIS (Sm.) Bréb.

Showing sheath stained with Methylene Blue and Picric Acid. Magnification 415 diameters. Photomicrograph with 100 watt condensed filament lamp, Wratten K_3 and B screens, 1/5" Objective, X10 Ocular, field and sub-stage condensers.

The usual methods of staining algal cells, when applied in this case with the hope of more clearly demonstrating the structure of this sheath, caused much distortion. In the summer of 1919 at the Marine Biological Laboratory, Woods Hole, Massachusetts, the writer worked out the following method for the use of the students, and as it has been tried out on subsequent occasions with uniformly satisfactory results, it is offered

[1] In this respect see Lütkemüller, J., Die Zellmembran der Desmidiaceen. Beiträge zur Biologie der Pflanzen, (Cohn), 8:347–414. 1902.

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as being suited for use with classes. The great abundance in which *Hyalotheca dissiliensis* (Sm.) Bréb. often occurs makes it peculiarly convenient, but the method is no doubt adaptable for use with other forms.

Fresh living material is placed in a .05% aqueous solution of Methylene Blue for 45 to 60 seconds. It is then removed, rinsed in distilled water and placed in a $\frac{1}{10}$ saturated aqueous solution of Picric Acid. This serves to fix the stain and brings out in a most striking manner the striations in the sheath. The material may be examined in the Picric Acid solution, or removed after a minute or two to water. Preparations are best used soon after staining, as the sheath begins to disintegrate after a few hours.

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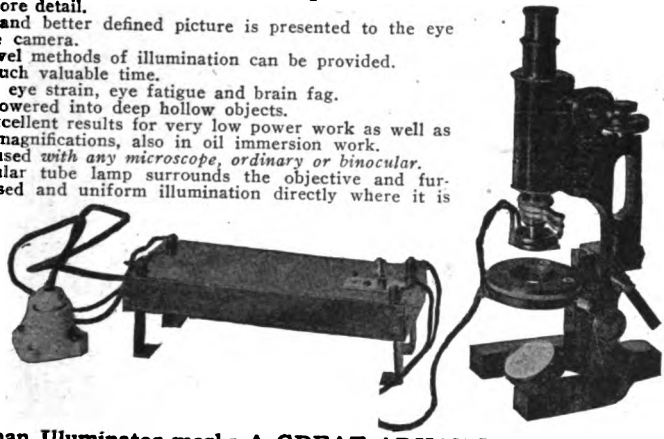

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INDEX TO ADVERTISEMENTS

Anglers Co., Chicago, Ill. III
Bausch and Lomb Optical Co., Rochester, N. Y. V
Comstock Publishing Co., Ithaca, N. Y. III
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JULY
1921

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TABLE OF CONTENTS

For Volume XL, Number 3, July, 1921

Observations on the Distribution and Life History of Cephalobium microbivorum Cobb and of its Host, Gryllus assimilis Fabricius, with one plate and three figures, by J. E. Ackert and F. M. Wadley 97

A Sarcophagid Parasite of the Common Field Cricket, by C. A. Herrick 116

Fresh Water and Marine Gymnostominan Infusoria, with four plates and three figures, by L. A. Hausman 118

Copper: Its Occurrence and Rôle in Insects and Other Animals, by R. A. Muttkowski .. 144

Department of Methods, Reviews, Abstracts, and Briefer Articles

Microscope Illumination with Reference to Brownian Movement and Combination Lighting, by A. Silverman 158

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)

Vol. XL JULY, 1921 No. 3

OBSERVATIONS ON THE DISTRIBUTION AND LIFE HISTORY OF *CEPHALOBIUM MICROBIVORUM* COBB AND OF ITS HOST, *GRYLLUS ASSIMILIS* FABRICIUS[1]

By
James E. Ackert and F. M. Wadley

Contents

Introduction 98
The Parasite, *Cephalobium microbivorum* Cobb 98
Description 98
Habitat 100
Methods of Procedure 101
Removal of Nematodes 101
Culturing of Nematodes 101
Observations on Development 102
Early Cleavage to Coiled Embryo 102
Hatching 103
Factor Affecting Development 103
The Host, *Gryllus assimilis* 104
Distribution 104
Habits 104
Habitats 105
Proportion of Male and Female Crickets in Nature 105
Distribution of *Cephalobium microbivorum* 105
Geographical Distribution 105
Distribution in the Cricket Hosts 106
Proportion of Sexes and Parthenogenesis 107
Effect of Nematode on Host 107
Life History of *Cephalobium microbivorum* 108
Life Cycle 108
Seasonal Endurance 109
Other Parasites of the Cricket 109
Summary 110
Literature Cited 112
Explanation of Plate 114

[1] Contribution No. 52 from the Zoology Department, Agricultural Experiment Station of the Kansas State Agricultural College.

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Introduction

While securing gregarines from black field crickets for class use, in October, 1918, the senior writer found heavy infestations of small nematodes, some of which were sent to Dr. N. A. Cobb for identification. Determining that these nematodes represent a new genus, Doctor Cobb suggested that studies be made on the distribution and life history of it. Observations on its distribution have been made at Woods Hole, Mass.; Falls Church, Va.; Douglas Lake, Mich.; Rockford, Ill.; and Manhattan, Kan. The studies on its life history and that of the crickets were made at Manhattan. Further work on certain phases of these studies would be very desirable, but as this cannot be done for some time, it seems best to put the present findings on record. The writers wish to express their indebtedness to Doctor Cobb for suggesting this nematode study, and to Director Frank R. Lillie of the Marine Biological Laboratory, Woods Hole, Mass., and Director George R. LaRue of the University of Michigan Biological Station for the privilege of using equipment at the respective stations.

The Parasite, Cephalobium microbivorum Cobb

Description

These nematodes which are from 2 to 3 mm. in length were identified as *Cephalobium microbivorum* n. g., n. sp., by Dr. N. A. Cobb, who submits this description.

The following characterizations and description, with figures, are taken from "Contribution to a Science of Nematology," No. IX; "*One Hundred New Nemas*," N. A. Cobb, 1920. The characters other than specific are assembled from Cobb's Keys.

Phylum Nemates

Subphylum Laimia: Nemas having a more or less distinct pharynx.

Class Anonchia: Nemas lacking onchia.

Subclass Anodontia: Nemas lacking odontia.

Order Polylaimia: Nemas having an unarmed pharynx, composed of two or more successive chambers more or less distinctly separated from each other.

Genus Cephalobium

Cavity of the pharynx more or less prismoid or cylindroid (not conoid or very irregular), and containing a glottoid organ at its base. Oesophagus with median bulb and posterior swelling. Amphids none so far as known. Seta-like labial papillae 6. Single lateral wing present; striae fine, plain. Spinneret absent.

Preanal and postanal papillae present on the male. Tail conoid or subconoid; terminus acute, unarmed. Bursa none. Spicula two, equal, more or less arcuate; not jointed; their width not uniform. Accessories (gubernaculum) present. Inner ends of spicula cephalated by constriction. Length of the spicula 1¼ times as great as anal body diameter.

54. *Cephalobium microbivorum*, n. sp. The single wing begins near the head and ends near the terminus. Its optical expression is either a pair of lines or a single line in the middle of a field one-twelfth as wide as the body. The contour of the body may become crenate in the anal region. There are about thirteen lateral organs on each side connected with pores

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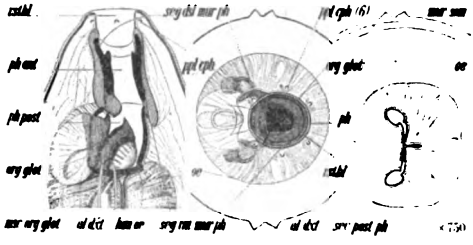
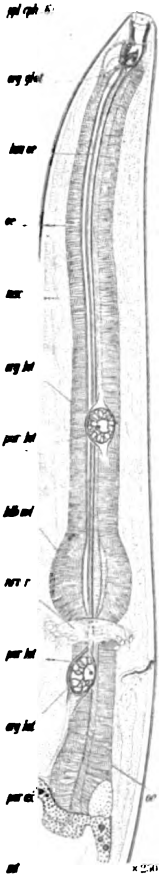
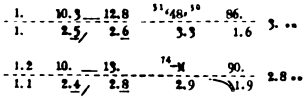
in the cuticle (see org. lat. fig. *54*). Base of the pharynx containing a large, complicated and peculiar dorsal glottoid organ (see fig. *54*). No amphids. The rather thin-walled intestine is set off by a collum one-eighth as wide as the neck, and has a rather distinct lumen. It becomes at once five-sixths as wide as the body, and in cross section presents two to four cells. From the somewhat depressed anus, the narrow, cutinized rectum extends inward and forward a distance one and one-fourth times as great as the anal body-diameter. Scattered yellowish granules of variable size occur in the cells of the intestine, the largest being one twenty-fifth as wide as the body; in addition, there are numerous very small granules. There is no tessellated effect. Subarcuate, conoid tail tapers from in front of the anus to the acute, fine terminus. There is no spinneret. From the elevated vulva, the rather small, somewhat weak vagina extends inward nearly at right angles to the ventral surface one-fourth the way across the body. Along the middle half of the body the two equal uteri contain thin-shelled, smooth, ellipsoidal eggs two-thirds as long as the body is wide, and appear to be deposited after segmentation begins. No embryos were seen in these eggs, only blastulas. For the most part the ova are arranged irregularly in the somewhat tapering ovaries. The two equal, rather strong, slender, arcuate, tapering subacute spicula are one and one-fourth times as long as the anal body-diameter, are more or less cephalated by constriction and when seen in profile have proximal ends nearly opposite the body-axis. Toward their distal ends four slender stiffening pieces are apparent. There is a simple, strong, and rather solid, straight accessory piece, one-third as long as the spicula, bending back from them at an angle of about 90 degrees, so that its proximal end lies opposite the body-diameter. There is no bursa. Near the beginning of the second quarter of the tail there is a pair of lateral pores similar to those on the female. Beginning just in front of the anus there exists on the tail a series of six submedian pairs of flattish-conoid, rather inconspicuous papillae. These occupy the anterior two-thirds of the tail, and have a formula as follows: 1 ( ) 1; 111; 1. The members of the posterior four pairs are located exactly opposite each other, the right hand member of each pair being slightly behind the left hand member. Papillae plainly enervated. Spicula conspicuous, rather close together; at the widest part about one-eighth as wide as the corresponding portion of the body, ending in minute "buttons." The lateral pores on the tail are the final members of the lateral series of lateral organs.







Habitat: Intestine of the field cricket, *Gryllus assimilis* Fabr. The males are considerably smaller than the adult gravid females.

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HABITAT

To ascertain the part of the cricket inhabited by *C. microbivorum* some crickets were carefully dissected. In each case the thin-walled ileum readily revealed the writhing nematodes which appeared in bold contrast to the dark fecal contents. None of these parasites was found in the coelom, nor in any organ outside of the digestive tract. In one case two dead adults were taken from the colon of a freshly dissected cricket, whose ileum contained several live specimens. To facilitate subsequent discussion a brief description and a diagram are given of the alimentary canal of the cricket host.

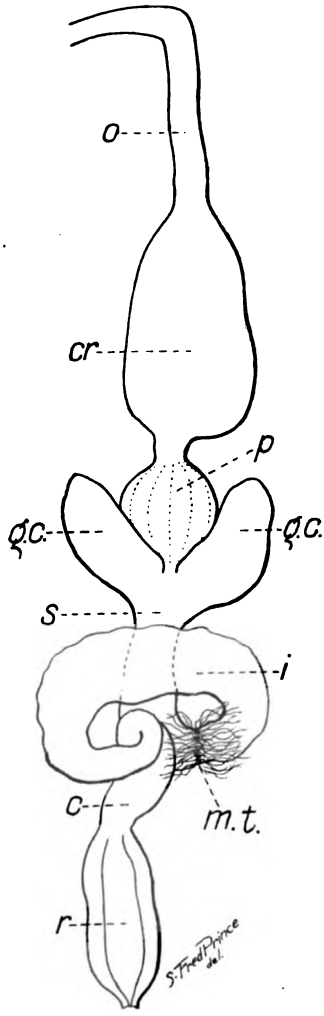

FIG. 1

Figure 1. Showing digestive tract of *Gryllus assimilis*. *c*, colon; *cr*, crop; *g.c.*, gastric caecum; *i*, ileum; *m.t.*, malpighian tubes; *o*, oesophagus; *p*, proventriculus; *r*, rectum; *s*, stomach. × 4.

The digestive tract (fig. 1) of this black field cricket, *Gryllus assimilis*, bears many resemblances to that of the large and nearly wingless western cricket, *Anabrus*, as shown by Packard (1878-79, p. 175). The oesophagus (o) connects with the mouth and after making a sharp bend proceeds through the posterior part of the head to open into the spacious crop (cr) which occupies the thoracic and anterior abdominal portion of the coelom. After greatly narrowing, the crop opens into the strong proventriculus (p) which is much larger than the corresponding organ in *Anabrus*. In *G. assimilis* the diameter of this organ exceeds three times that of the junction with the crop, whereas in *Anabrus* the proventriculus is considerably reduced in size.

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The proventriculus opens by a very narrow canal into the true stomach (s) which immediately gives off anteriorly two large, flattened gastric caeca (g.c.), situated one above the other. The stomach is surprisingly slender. After passing backward a short distance it makes an abrupt turn upward, narrows slightly and terminates, giving off numerous Malpighian tubes (m. t.) where it joins the intestine. Like *Anabrus* the remainder of the digestive tract is distinctly divisible into three portions: the ileum, colon, and rectum. Unlike the large western species, however, the stomach of *G. assimilis* is considerably shorter and the ileum (i) longer and much more capacious. The walls of the stomach are thick and translucent, while those of the ileum are thin and transparent. So thin are the walls of the latter that not only are the enclosed, motile nematodes visible, but also the eggs in the females' bodies. Before terminating, the ileum narrows considerably, makes a pronounced twist and then opens into the larger, thick-walled colon (c). Continuing posteriorly, the colon with a slight constriction connects with the still larger rectum (r) with which the anus communicates.

Methods of Procedure

REMOVAL OF NEMATODES

The principal method employed for the removal of the nematodes is here briefly described. After excising the head of the cricket an incision was made in the posteroventral wall of the abdomen with fine scissors. By cutting forward through the median ventral surface, being careful not to cut deeply, nearly the whole lower body wall could be laid open without disturbing the digestive tract. On inserting fine forceps into the mesothoracic region the crop could be seized, and with slow, sustained effort the entire digestive tract withdrawn. In this extended condition the food tube was placed upon an ordinary glass slide, the rectum being excised at the anus. After covering with a few drops of normal salt solution, the intestine was teased with needles. The optical examinations were made with the aid of dissecting, binocular, and compound microscopes.

CULTURING OF NEMATODES

The culturing of the nematodes and their eggs was carried on for a time with fair success, but this phase of the problem should be continued. Having the nematodes on regular microscopic slides made it easy to remove the substances not wanted in the culture, and to add materials desired. With a little care the culture could be held to a restricted area on the slide by the surface tension of the solution. To prevent drying, the culture slides were placed in Petri dishes containing a few drops of distilled water. Subsequent microscopic examinations of the cultures were made either in or out of these small moist chambers.

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Culture fluids used included normal salt solution alone, with fecal material, with peptone, and with both fecal material and peptone. Eggs hatched in each fluid, but growth of young nematodes appeared to occur only when a few drops of 0.8% peptone (in distilled water) were added. Dilute peptone was one of the successful solutions used by Welch and Wehrle (1918, p. 151) in their extensive nematode cultures.

In normal saline solution adult nematodes lived from one to six days, eggs developed and hatched, but even vigorous free embryos failed to increase in length and died within three days. When a few drops of 0.8% peptone were added to normal saline, embryos hatched and lived six days. The best results were obtained with equal volumes of normal saline and 0.8% peptone solution and a trace of cricket feces. In this medium several free embryos lived eight days, and a few thirteen days, the latter increasing their body lengths 16⅔ per cent. However, before the young nematodes had developed markedly, the culturing had to be abandoned on account of failure to secure nematodes. The adult crickets which had been collected prior to a cold wave succumbed in a few days, thus destroying the source of supply.

Observations on Development

Early Cleavage to Coiled Embryo

The observations on development were upon living material, no attempt being made to trace the formation of germ layers or organs. The translucency of the dividing cells made it possible to follow the individual blastomeres until they formed a more or less spherical mass, such as shown in fig. 6. Whether such developing forms as represented in figs. 6 and 7 were hollow is uncertain, owing to the growing opacity of the embryos. For convenience in description the terminology of Martin (1913) is partly followed. For the uncertain stages shown in figs. 6 and 7, *a* morula rather than *the* morula is used. Likewise, when the vermiform shape of the embryo is first attained (figs. 8, 9) the term *curved* embryos is employed, whereas, the fully attenuated enclosed embryo (fig. 11) is designated as a *coiled* embryo.

These few observations on the development of the external form of *C. microbivorum* are given in the hope that someone may find opportunity to work out the embryology of this nematode. Such a study would be valuable and would be greatly facilitated by the thin, elastic egg shells.

In the present studies fertilized eggs, which can be distinguished by their clear nuclei, were mounted under cover slips in normal saline solution and studied under low and high powers of the microscope, a few drops of distilled water being added occasionally to compensate for evaporation. In other cases the live females containing eggs were so mounted, and in

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this way external development was traced from the fertilized egg through hatching.

Under these conditions the early cleavage stages, as shown in figs. 2 to 5, develop somewhat rapidly, each cell division occurring in from ten to sixteen minutes. As segmentation proceeds and the bulk of the embryo increases, the thin, elastic shell expands accordingly. Eggs in an early cleavage stage (fig. 5) at 5 p. m. were in a morula stage with large blastomeres (fig. 6) at 8 p.m. Eighteen hours afterward development had proceeded to a morula with small blastomeres (fig. 7), in twenty more hours to a curved embryo (fig. 9), and six hours later to a coiled, motile embryo (fig. 11).

HATCHING

The stages represented in figs. 2-11 inclusive were observed in the uteri living worms mounted in normal salt solution under cover slips. Hatching occurred only after the eggs were ejected. Emergence from the egg was accomplished by repeated thrusts of the anterior end of the embryo against the thin shell which soon began to give way (fig. 12), finally rupturing (fig. 13) and liberating the writhing embryo.

FACTOR AFFECTING DEVELOPMENT

As noted above, eggs in nematodes which were in culture media developed somewhat rapidly, attaining the coiled, motile stage in approximately two days. But in the body of the live host uterine eggs do not appear to to develop beyond the four-cell stage, as numerous examinations have shown. However, such eggs in a dead and somewhat macerated cricket were in more advanced stages of development. Also in the voided feces of an infested cricket, eggs in a morula stage were found. Thus it appears that the failure of uterine eggs to develop beyond the four-cell stage in the living cricket is due to an inhibiting factor. This factor the writers believe to be lack of sufficient oxygen. In the higher animals it is well known that the intestines contain enormous numbers of bacteria which must take much of the free oxygen, especially in the colon and adjacent regions where anaerobic bacteria thrive. In the ileums of these crickets there were numerous bacteria. The cases of advanced development of uterine eggs of the nematodes in the macerated cricket would favor this interpretation, as the thin wall of the disintegrating ileum quickly ruptures, admitting oxygen. Likewise, eggs which developed up to hatching in the uteri of cultured nematodes would be in the presence of more oxygen than when in the ileum of the cricket. An ample supply of oxygen to eggs in the separate fecal pellets is obvious. That additional oxygen accelerates development in nematode eggs has been determined by the senior writer

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who observed more rapid development in cultured (normal saline) eggs of *Ascaridia perspicillum* on the addition of 10% hydrogen peroxide (unpublished results).

The Host, Gryllus assimilis

DISTRIBUTION

In October, 1919, when the cricket examinations were at their height some adults were sent to Mr. James A. G. Rehn, who identified them as *Gryllus assimilis* Fabricius. Rehn and Hebard (1915, pp. 295, 296) regard this species as the common black field cricket of the Americas, which ranges from Canada to Argentina and from the Atlantic to the Pacific.

In the vicinity of Manhattan there are two races of this species, one maturing in August and September, the other in April and May. The fall adults lay their eggs in October, depositing them in the soil, under stones, and in other protected places. These eggs hatch the following spring according to Bruner (1886, p. 194), and the young mature in August and September. Concerning the occurrence and behavior of the spring adults, a few notes from the senior author's records for another problem are given, "About the last of May, 1915, adult crickets were found in nature mating. Several pairs of these were placed in cages containing sterilized earth, some carefully selected stems of alfalfa, and a small block of wood. Care was taken not to introduce any other animals." These records show that every second day the caged crickets were given one of the following foods: green alfalfa, fresh apple, algae, and small bits of fresh beef. Young crickets hatched in three weeks. By October the nymphs were approximately half-grown, averaging one-half the length of the adults.

From these and other observations it appears that in the vicinity of Manhattan, Kan., *G. assimilis* produces only one brood per year, but is represented by two races, the fall adults, laying their eggs in the autumn, passing the winter in this stage, hatching in the spring, and maturing in late summer or autumn; and the spring adults, depositing their eggs in the spring, hatching in early summer, passing the winter in a nymphal stage, and maturing the following spring. These respective findings are in close accord with the observations of McNeill (1891, p. 5) for *G. abbreviatus* in Illinois and of Blatchley (1901, p. 439) for *G. pennsylvanicus* in Indiana.

HABITS

Concerning the habits of the common black cricket, Blatchley (1901, p. 436) states that it is nocturnal, omnivorous and cannibalistic. The present studies indicate that these crickets in nature are largely nocturnal, but that they may stridulate, move about, and feed to some extent in the day time.

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That they are omnivorous in nature is amply confirmed by these observations. Plants on which they have been seen feeding include alfalfa (*Medicago sativa*), bluegrass (*Poa pratensis*), bindweed (*Convolvulus spp.*), crabgrass (*Syntherisma sanguinale*), and Bermuda grass (*Capriola dactylon*). Decomposing plant and animal tissues appear not to be distasteful, as the crickets have been seen feeding on both. Portions of dead crickets and other arthropods have been taken in preference to wilted grass, and in a few instances the animal tissues were in an advanced stage of decomposition.

Cannibalism is of frequent occurrence among the common black crickets, but apparently they seldom attack each other in life. In ordinary captivity mortality is high; some of the captives usually survive and frequently feed upon their deceased mates. The senior writer in connection with another problem reared crickets from eggs in large life history cages, making almost daily observations for months. Crickets were often seen dying, and sooner or later others began to devour them. In a single instance, a live cricket was observed to approach a dying one, lying on its back, and begin feeding on the latter's hind femur. At no other time, either in 1915-16 or during the present studies, has one cricket been seen to feed upon another living one.

HABITATS

The wide distribution of *G. assimilis* is doubtless due in part to its omnivorous feeding habits and to its varied habitats. Among the habitats from which it has been taken are the following: at edges of side walks, in holes in the ground and chinks in walls of buildings, under old hardened ox feces, sticks, boards, logs, stones, and stone walls, and among various kinds of vegetation.

Besides proximity to food and a reasonable amount of protection the diurnal habitat of this cricket must afford an atmosphere of comparatively high humidity. In artificial rearing the mortality was exceedingly high until water was sprinkled into the cages, when the percentage of survivals markedly increased.

PROPORTION OF MALE AND FEMALE CRICKETS IN NATURE

The writers found the number of male and female crickets to be approximately equal in nature, except during late October after the breeding season is over. At this time the adult females, with their abdomens distended with eggs, far outnumbered the surviving adult males.

DISTRIBUTION OF CEPHALOBIUM MICROBIVORUM

GEOGRAPHICAL DISTRIBUTION

Examinations of black field crickets for *C. microbivorum* have been made in five states: Kansas, Massachusetts, Virginia, Michigan, and

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Illinois, but to date these nematodes have been found only in Kansas and Virginia. From April to June, 1919, Dr. N. A. Cobb examined a few black field crickets at Falls Church, Va., and found *C. microbivorum* in nearly every cricket. At Manhattan, Kan., these nematodes are known to have been of common occurrence in the adult black crickets during the autumns of the last three years (1918, 1919, 1920).

DISTRIBUTION IN THE CRICKET HOSTS

A study of the data collected during the search for *C. microbivorum* in the local black field crickets reveals some interesting points in the distribution of these nematodes in their hosts. Most of the examinations of the crickets were made during the periods between September 19 and October 31, in 1919 and 1920. From Table I it is seen that the number of female crickets examined exceeds that of the males. This was due to certain collections made late in October after the breeding season and after the consequent heavy mortality of male crickets. Collections made in September included nearly equal numbers of males and females.

TABLE I. SHOWING NEMATODE INFESTATION OF ADULT CRICKETS EXAMINED BETWEEN SEPTEMBER 19 AND OCTOBER 31, 1919 AND 1920, AT MANHATTAN, KAN.

	No. Crickets Examined	No. Crickets Infested	Per cent Infested	Total No. Nematodes	Average Infestation per Cricket	Range of Infestation
Male Crickets	14	10	71.4	217	21.7	2 to 51
Female Crickets	33	30	90.9	822	27.4	3 to 91
All Crickets	47	40	85.1	1,029	25.7	2 to 91

From Table I it is seen that over eighty-five per cent of the crickets examined by the writers between September 19 and October 31 were infested with this nematode. Approximately seventy per cent of the males and ninety per cent of the females contained these parasites. Table I likewise shows that both the range and average infestation of the females exceed that of the males. That the intestine of the male cricket furnishes a suitable environment for these parasites is evident from infestations amounting to as many as fifty-one *C. microbivorum*. Consequently the explanation of these phenomena must be sought elsewhere. In October the females' bodies are usually gorged with eggs, and are larger than those of the males. Obviously, to afford this greater development, more food

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would be required than for the males, thus increasing the chances of the females ingesting a larger number of nematode eggs or larvae. The large, distensible crop (fig. 1, cr) is adapted for receiving quantities of food, and the numerous fecal pellets voided daily by these females are evidences of large appetites. Thus, to the writers, the most plausible explanation of the higher percentage, average, and range of nematode infestations in the females is that the engorged females take more food, and thus, on the average, swallow more eggs or larvae.

Occasional examinations of three or four specimens of *G. assimilis* were made during June, August, and September, 1920. No specimens of *C. microbivorum* were found in any of these crickets until August 21 when one was taken from an adult female. Of six mature crickets--three males and three females—examined on this date, two of the females contained nematodes, the other infestation consisting of two immature specimens. In September the recorded infestations ranged from seven to thirty-one nematodes, and in October from seven to as many as ninety-one.

In addition to the examinations of adult crickets shown in Table I, some half-grown black crickets were dissected on November 3, 1920. In the ileum of one of these nymphs were two mature specimens of *C. microbivorum*. The significance of this observation will be discussed later.

PROPORTION OF SEXES AND PARTHENOGENESIS

The nematodes observed included noticeably more females than males, and in one case males were entirely lacking. This case will be discussed presently, but concerning the presence of more female nematodes than males, the writers have no explanation to offer. Merrill and Ford (1916, p. 127) likewise found the females more numerous in the two species of nematodes they studied.

In the case just mentioned in which males were lacking, the infestation consisted of three females each containing fertilized eggs. These females may have been fecundated by males which had already left the host, as two dead nematodes were found in the colon of a cricket immediately after killing. Or, the females in question may have been parthenogenetic as Welch and Wehrle (1918, p. 159) and others have observed in small nematodes. It is possible also that they may have been protandric hermaphrodites, but the almost constant occurrence of males in the infestations favors the view that the females were probably fecundated by males which subsequently passed from the cricket.

EFFECT OF NEMATODE ON HOST

The effect of *C. microbivorum* on the host does not appear to be serious, as apparently normal crickets often harbored thirty or more of these nematodes. On the other hand, it seems probable that so many compar-

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atively large entozoa must be detrimental to the host. Flury (1912) has shown in the cases of nematodes parasitic in higher animals that they cause injury not only by taking food material and by stoppage, but that on account of their imperfect digestive system their excreta contain toxins which are absorbed by the host. Some of these injuries would be likely to occur in the infested crickets.

Life History of Cephalobium microbivorum

Life Cycle

As stated elsewhere, *C. microbivorum* matures in the intestine of the field cricket and eggs normally develop to the four-cell stage. When such eggs are ejected and kept moist development proceeds to the coiled, motile embryo stage in about two days. Cricket feces voided during the night and examined the following morning contained eggs of *C. microbivorum* in a morula stage. To ascertain the probable fate of such eggs in nature, four adult female crickets were placed in a lantern globe cage over some moist, sterilized earth. They lived from four to six days, the dead ones being removed before they were attacked by their mates. Two of these crickets were subsequently found to be infested with mature female nematodes containing eggs. The earth in the cage was moistened nearly every day, but as it was kept uncovered in the laboratory, evaporation was rapid and the culture dried out several times. Ten days after the infested crickets were placed over the sterilized earth examinations of portions of the latter were made which revealed two dead larvae, slightly larger than newly hatched embryos.

These observations indicate that in nature the eggs pass from the body of the cricket in early cleavage stages, and since the diurnal habitat of the cricket must have an atmosphere of comparatively high humidity, as shown elsewhere, a fair percentage of the nematode eggs voided in the daytime would be protected by the humidity of the cricket habitat. Since in culturing, these nematode eggs hatched in each separate medium, it is logical to infer that they would hatch in the moist débris of a cricket habitat. The thin elastic shell which bursts after a few thrusts would probably not long confine the embryos. Another reason which leads the writers to think that these young nematodes pass a period free in nature is that while immature stages of *C. microbivorum* are found in the ileum of the cricket, these nematode larvae are always much larger than the newly hatched embryos. If infection were caused by the cricket's ingestion of nematode eggs, one would expect occasionally to find in the crickets young nematodes the size of newly hatched ones, but this has failed to occur in the removal of over one thousand of these nematodes. The writers made no attempt to infest crickets by giving them hatched embryos or free larvae, but Merrill and Ford (1916, p. 127) succeeded in infecting termites with free larvae of the nematode, *Diplogaster aerivora* Cobb.

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The indiscriminate feeding habits of the crickets would give ample opportunity for the ingestion of larvae of *C. microbivorum*, for as stated elsewhere, they feed upon decomposing plant and animal tissues, and these substances are commonly found in the moist diurnal habitats. Once in the ileum of the cricket the young *C. microbivorum* evidently thrive, for they occur in active stages ranging from one-third to normal lengths.

SEASONAL ENDURANCE

In the light of the information available, this nematode's problem of enduring the seasons seems relatively simple. It has been noted that in the vicinity of Manhattan, Kan., two races of *G. assimilis* occur, one spending the winter in the egg stage, and the other in the nymphal stage. The finding in November of mature specimens of *C. microbivorum* in nymphal black field crickets which live over the winter here and elsewhere in protected places indicates one way in which the cold season may be endured. These nymphal crickets mature in May, and deposit their eggs early in June, thus making it possible to shelter these adult parasites until summer.[2] By this time the eggs of the other race are hatched, furnishing possible hosts for the young *C. microbivorum* liberated as eggs from the winter-enduring nymphal crickets. Protection of the larvae against sudden desiccation and high temperatures would be afforded deeper in the habitat. At any rate, the fact that the fall infestations are the heaviest indicates that this nematode's problem of enduring the summer is not a serious one.

Another possible means of *C. microbivorum* enduring the winter might be afforded by the habit of the fall adult crickets crawling into the ground and into other protected places when their life work is finished. If such infested crickets were killed by freezing and remained congealed throughout the winter, the spring season might be well advanced before maceration of the crickets' bodies proceeded far enough to admit sufficient oxygen for the development of the enclosed nematode eggs. The plausibility of this method is strengthened by the fact that many nematodes can withstand some freezing. It is possible, of course, that larvae of these nematodes pass the winter free in the soil. Further studies in connection with the culturing of *C. microbivorum* will doubtless settle this and other points in its life problems.

OTHER PARASITES OF THE CRICKET

While searching for *C. microbivorum* in the crickets certain other parasites were encountered; viz., gregarines, gordiacea larvae and dipterous larvae. In July, 1919, gregarines were present in many of the common black crickets examined at Woods Hole, Mass. At Douglas

[2] The writers made no examinations of the spring adults, but Doctor Cobb's examinations from April to June were of adults of this race in nearly all of which he found *C. microbivorum*.

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Lake, Mich., these protozoan parasites were of frequent occurrence in crickets in August, 1920, and larval gordiacea were occasionally found.

Of the 106 mature or nearly mature crickets examined gregarines were found in thirty-seven per cent, and gordiacea larvae in 9 per cent of them. The crickets were taken from four localities; viz., Douglas Lake shore line about seventy-five yards wide; Burt Lake shore line approximately ten yards in width; Sedge Pool shore line about eight yards in width; and an upland pasture one and one-half miles from water.

The only gordiacea infestations occurred in the Sedge Pool locality, which was also the most favorable for gregarine infestations. This pool, which is separated from Douglas Lake by a narrow ledge, is approximately 200 feet long by 120 feet wide. It is protected by two to four foot banks and by a substantial growth of timber on three sides, leaving the east and southeast sides open to the direct rays of the sun. Most of the crickets were taken on the narrow ledge at the east side of the pool within ten to twenty feet of the protected water's edge. The infested crickets contained gordiacea larvae in later stages of development, some of them having attained the dark adult coloration. Two that escaped from a cricket in a bottle of water were readily identified as *Paragordius varius* (Leidy). This was the only species obtained by May (1919), who examined several hundred crickets from the east shore of Douglas Lake in connection with his studies on the life history of this species. The lowest percentage (30 per cent) of gregarine infestation was in the upland pasture one and one-half miles from Douglas Lake, while the highest (45 per cent) occurred at Sedge Pool. This indicates that these protozoan parasites thrive better under moister conditions.

Records for gregarine infestation of the crickets examined at Manhattan, Kan., are not complete, but of twenty crickets taken from nature between June 24 and October 21, 1920, eleven, or fifty-five per cent of them, were infested. The infestations ranged from one to as high as 517 gregarines, the average being slightly over sixty-two. Not a specimen of *Paragordius* was found here, but from two crickets examined by Herrick (1921) a few sarcophagid larvae were taken, this apparently being a new case of parasitism in *G. assimilis*.

Summary

1. In the autumns of 1918, 1919, 1920 black field crickets, in the vicinity of Manhattan, Kan., were infested with a new species of nematode which has been identified as *Cephalobium microbivorum* Cobb.

2. In the body of the living cricket development of the eggs has not been observed to exceed the four-cell stage. This is attributed to lack of sufficient oxygen.

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3. In culturing, eggs hatched in all moist media used, but young nematodes grew only when 0.8% peptone was added, two specimens increasing their lengths 16⅔ per cent in thirteen days. In early cleavage, each cell division was accomplished in from ten to sixteen minutes; and in approximately two days the embryo was fully formed. Hatching is accomplished by repeated thrusts of the anterior end of the embryo against the thin elastic shell which soon ruptures and liberates the embryo.

4. The parasitic habitat of this nematode is the spacious ileum of *Gryllus assimilis* Fabricius, which is the common black field cricket of the Americas, ranging from Canada to Argentina and from the Atlantic to the Pacific. In the vicinity of Manhattan, Kan., this species is represented by two races, one maturing in August and September, the other in April and May; each race produces one brood of crickets per year. The race maturing in the spring winters here in the nymphal stage, while the adult fall race spends the cold season in the egg stage. Eggs of *G. assimilis* hatched (June) in three weeks after deposition. Late in October they were half grown, and by the last of the following May they were mature and mating.

5. These crickets are omnivorous, feeding on various kinds of plant and animal tissues, both fresh and decomposed. Cannibalism is of common occurrence among them. They are known to devour their dead and dying, but not to attack each other in normal condition. Their diurnal habitats, which may include a variety of situations. must furnish some protection and sustain a certain amount of moisture.

6. The numbers of adult male and female crickets observed in nature were about equal, except in the autumn after the breeding season, when more females survived.

7. Of crickets examined in five states, only those from Kansas and Virginia have been infested with *C. microbivorum*. At Manhattan, Kan., about 85 per cent of the fall adult crickets examined were infested; 70 per cent of the males and 90 per cent of the females. The females also contained a larger number of these parasites. Both the higher percentage of parasitism and the heavier infestations of the females are attributed to their greater voracity.

8. Infestations of fall adults were first found in August, the parasites being young and few. By September adult nematodes were taken and the size of infestations increased to 31. In October both young and adults were numerous, a maximum infestation amounting to 91 of these nematodes.

9. Nymphal, black field crickets of the race which winters in this stage were infested with mature nematodes in November.

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10. Female nematodes were more numerous than males; for this phenomenon no explanation is offered. One cricket contained 3 female nematodes, each having fertilized eggs. Death of the males after fecundation is deemed more probable than parthenogenesis or protandric hermaphroditism.

11. No positive deleterious effect of the parasites on the host was observed, but this does not preclude possible injury.

12. The life cycle of *C. microbivorum* appears to be as follows: The nematode matures in the ileum of the common field cricket. Its eggs are deposited in early cleavage stages and passed from the body of the cricket. Under moist conditions furnished by the diurnal habitat the eggs soon hatch, and in the presence of nutritive substances the liberated embryos grow. Sooner or later the larval nematodes are swallowed by the omnivorous cricket in whose ileum they mature.

13. The nematode's problem of enduring the seasons is apparently solved by the occurrence of the two races of *G. assimilis*, the winter nymphs sheltering some of the mature nematodes through the colder months and the young of the fall adults ingesting larval nematodes during the warmer ones.

This method is probably supplemented by the infested bodies of certain fall adult crickets which, though dead, pass the winter in a somewhat congealed condition, the macerating bodies later liberating mature nematodes and eggs.

14. Other parasites encountered in the crickets examined included gregarines, gordiacea larvae and dipterous larvae. Gregarines were found generally, *Paragordius varius* larvae only at Douglas Lake, Mich., and sarcophagid larvae only at Manhattan, Kan.

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Explanation of Plate

All drawings were made with the aid of a camera lucida and are of the same magnification, X 400. The figures show stages in the embryological development of the external form of *Cephalobium microbivorum* Cobb.

Figs. 2 to 5. Eggs in early cleavage.
Fig. 6. Morula with large blastomeres.
Fig. 7. Morula with small blastomeres.
Figs. 8, 9. Eggs containing curved embryos.
Figs. 10, 11. Eggs containing coiled embryos.
Figs. 12, 13. Eggs in process of hatching.

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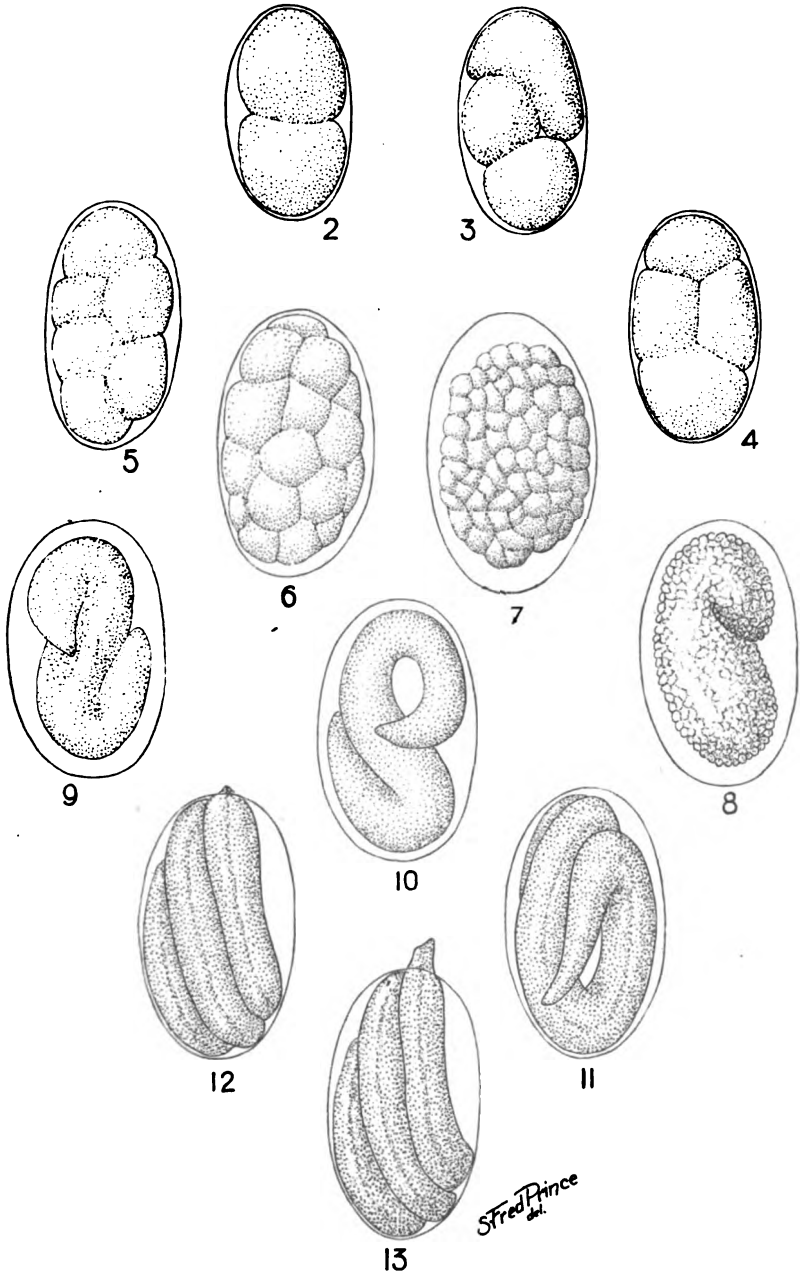

PLATE IV

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A SARCOPHAGID PARASITE OF THE COMMON FIELD CRICKET[1]

By

Chester A. Herrick

The purpose of this article is to record what seems to be a new case of parasitism in the cricket, *Gryllus assimilis* Fabricius, which, according to Rehn and Hebard (1915, pp. 295, 296), is the common black field cricket, not only of Kansas but of America. The cricket hosts of this parasite were taken in an alfalfa field near a stone wall. This wall and the débris along the south side of it afforded excellent protection for the congregated crickets. Numerous dead crickets were in the wall and under the débris near it and may have attracted the sarcophagids.

While examining these black field crickets for nematodes under the direction of Dr. J. E. Ackert, two insect larvae were found on Sept. 29, 1920. From the method of examination it is apparent that these larvae were in the body-cavity of the crickets. After excising the extreme posterior end of the cricket the thorax and abdomen were gently separated, and by sustained effort the whole intestine was removed from the abdomen. While the intestine was being examined the active larval parasites escaped through the large open end of the abdomen. One larva was 3 mm. in length, while the other, which was further developed, was 14 mm. long. The large larva was placed in a small covered tin box where, within 24 hours, it had pupated and become cemented to the floor of the box.

In this container the pupa was kept at laboratory temperatures, which ranged from 55° to 96° F., with an average temperature of 72° F. On the nineteenth day after pupation the adult fly emerged. It was sent to the Bureau of Entomology of the United States Department of Agriculture, where Dr. J. M. Aldrich identified it as *Sarcophaga kellyi* Aldrich.

This sarcophagid was first seen by Kelly (1914), who discovered it as a parasite of grasshoppers at Wellington, Kan., in mid-summer. His attention was attracted to certain flies that struck flying grasshoppers and caused them "to drop to the ground as if shot." On examining such grasshoppers he found tiny larvae crawling toward the base of the unfolded hind wing. Similar observations were subsequently made in New Mexico by Smith (1915), who found that this fly chose healthy, freshly molted, or inactive grasshoppers for the deposition of its larvae. He states (p. 8) that, "The female (*Sarcophaga kellyi*) upon locating a suitable victim was observed to alight upon the dorsum of the thorax and quickly deposit sev-

[1] Contribution No. 29 from the Zoology Department, Agricultural Experiment Station of the Kansas State Agricultural College.

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eral living maggots, which, encountering only the soft tender membrane, speedily made their way into the body cavity of their host. The maggots are capable, however, of entering a host which is fully dried out and hardened." This observer found as many as sixteen of these larvae in the body-cavity of a single grasshopper. He found that the larvae usually escaped through the wall of the thorax immediately behind the anterior coxa, but that others either bored through the abdominal wall or escaped through the anus.

Kelly (p. 438), who reared large numbers of *S. kellyi*, found that the larvae deposited in the fall, on escaping from the grasshopper host, penetrated the ground to a depth of from one-half an inch to 2 inches, where they remained throughout most of the winter. Pupation in nature occurred early in March and adults emerged from late March until the last of May. In warm weather Kelly (p. 439) found that the life cycle was completed in much shorter periods. By June or July a second generation had matured, and from this time "until November no distinction could be made between generations on account of overlapping. However, judging from the rapidity of their development, there were probably three or four additional generations, making about five or six for the season." According to this author, *S. kellyi* has been reared from grasshoppers at Wellington, Kan., Washington, D.C., and points in New Mexico, Arizona, and Utah.

Summary

1. Larvae of *Sarcophaga kellyi* Aldrich were found inhabiting the body-cavity of black field crickets, *Gryllys assimilis* Fabricius at Manhattan, Kan., in September, 1920.

2. This seems to be a new case of parasitism in the black field cricket.

3. The larvae of *Sarcophaga kellyi* have been reported from grasshoppers in Kansas, Washington, D.C., New Mexico, Arizona, and Utah.

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FRESH WATER AND MARINE GYMNOSTOMINAN INFUSORIA

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INTRODUCTION

The present contribution to the survey of the protozoa deals with the characteristic appearance, habits, and habitats of members of one of the largest and most important groups of the protozoa, namely, the *Gymnostomina*. Within this suborder are included many of the largest of these unicellular forms of animal life; forms which constitute one of the most, if not, indeed, the most important source of food supply for the smaller aquatic organisms, which in their turn form the bulk of the food of fishes. Their presence in ponds and streams is of great importance, for they convert refuse matters which might pollute the water into an available source of food for higher forms of life. A study of the protozoan faunas of waterways should, it seems, go hand in hand with a study of the problems of water purification, and of the preservation and utilization of our aquatic resources.

The majority of the species of *Gymnostomina* treated in this paper are fresh water. Several marine species are also included.

The water samples of which examination was made were secured from various portions of New York, Connecticut, Massachusets, and Mississippi, and over 1,000 were examined. They were taken from open lakes, ponds, roadside pools, rivers, brooks, rills, marshes, watering troughs, and the like.[1] The marine samples were secured from the Connecticut shore of Long Island Sound and from tidal estuaries and embayments, in the vicinity of New Haven.

METHODS OF STUDY

Methods of collecting material containing protozoa, in the field, are too well known to need much discussion here. The methods used in the present investigation underwent no decided original modifications from the methods commonly employed.[2]

Half a dozen pint fruit jars, fitted into a small, suit-case-like conveyance, together with a small silk plancton net, a large, long handled cooking spoon, and several glass tubes of various lengths (with detachable compression bulbs for "sucking"), comprised the entire field equipment. The jars were labelled, and a record kept of the nature of the locations from

[1] See Hausman, L. A. Observations on the Ecology of the Protozoa, Am. Nat., vol. 4, 1917, p. 157.

[2] See Hausman, L. A. A Contribution to the Life History of *Amoeba proteus*, Leidy, Biol. Bull., No. 5, May, 1920, p. 340.

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which the samples were taken, for future reference. Likewise each precise spot whence samples came was indicated on a topographic map. Possibly this may be found useful at some later time.

Upon arrival at the laboratory the samples were transferred to wide, open-mouthed jars. An examination was made of each sample immediately, and for a week or so, on each succeeding day, with the view of keeping record of the new species which emerged from encystment with the gradual stagnation and putrification of the water, for except in a very few cases the samples contained algae or other vegetal matter. The small bolting silk net shown in Fig. 3 was used for concentrating the infusoria content of one or more pipettefuls of water from the middle or bottoms of the samples where the water was usually more or less clear. No concentration methods were needed in the examination of the surface scum of the putrescing material.

All measurements were made with an ocular micrometer, or from a ruled millimeter slide, from retarded living, or freshly killed specimens.

The characters which are, perhaps, the most satisfactory for use in the identification of the living animals are: the contour of the body, the positions of the buccal cavity and of the largest contractile vacuole, and the disposition of the cilia. Killing and staining, or intra vitam staining may make apparent the structure of the pharynx and also of the nuclear elements. This treatment may sometimes be necessary for bringing out of the cilia. Methods of post mortem and of intra vitam staining will be discussed later.

For the first examination of samples a small drop of water was taken from the top scum, or from concentrated material (the results of straining) and mixed with an equal volume of very viscous gelatine solution, and the whole thoroughly stirred together on the slide with a curved needle.[3] Or often several drops were mixed with an equal part of the gelatine in a watch crystal and used on the slide when needed. The drop on the slide was now carefully flattened out and examined *without a cover glass* under low power (16 mm. objective and 4x eyepiece) to ascertain if the solution were of a viscosity great enough to check sufficiently the movement of the protozoa. If not, it was allowed to concentrate still more by evaporation, until properly viscous, and a cover glass applied. Magnification with the 16 mm. objective and the 4x and 10x eyepieces, and with the 4 mm. objective, and the same two eyepieces was usually found of sufficient strength for the determination of the species described in this paper. A word of caution is to be given here concerning the clarity of the gelatine solution. The gelatine used must be of the best grade and the solution must be

[3] See Hausman, L. A. The Manipulation and Identification of the Free-Swimming Mastigophora of Fresh Waters, Am. Nat., vol. 44, 1920, p. 333.

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perfectly fresh. It was found that gelatine which had stood for some time became cloudy in appearance and stringy in texture, due to the growth of colonies of mould plants and bacteria.

Another method of quieting the movements of the protozoa, which was developed, consisted in chilling the slide and its supported water drop on a small block of ice.[3] As the temperature decreased the motions of the protozoa became slower and slower, though never so slow as those incarcerated within the gelatine mixture. This method was devised more in the spirit of curiosity than in any hope that it would be as great an aid as the gelatine method of quieting movement.

Permanent mounts of the infusoria are believed to be very unsatisfactory, with the exception of those made of *Difflugia*, *Arcella*, *Euglypha*, the *Foraminifera*, and others whose bodies secrete a protective shell or test. And here it is the test and not the creature itself which is preserved in its original form. During the process of killing, of staining, and of mounting, the body form is more or less distorted, and the cilia deformed or lost. The most convincing demonstration of the poverty of the mounted slide can be had by examining together a living *Paramoecium* retarded in the gelatine solution, or one freshly stained intra vitam, and a mounted slide, of the same creature, of the best manufacture obtainable. For optimum results in the study of gross anatomy, at least, or for the needs of the systematist, nothing, I think, can equal the intra vitam staining, with the creature hampered in its movements in the gelatine solution. The movements of the cilia or of the contractile vacuoles are often of the greatest aid in determining their position and form. In fact the presence and form of the pharynx in its entire length can often be made out, in certain species, only by means of the cilia vibrating within it.

The stains[4] most frequently used were methyl blue, and gentian violet. Safranin, methyl green, and iodine were also used. Safranin, it was found, stained the deepest, and methyl blue the least. For certain forms, therefore, the one was used, and for others, the other. In the case of each stain a 95% alcoholic solution of the dry stain was made and kept in a small bottle ready to be diluted before applying to the slide. The staining set holder (Fig. 1) was designed to contain in a compact and convenient form the requisite number of stains, and other reagents, together with solid glass dipping rods for each. Thus any mixture of reagents was prevented. The labels (shown underneath the holes for containing the dipping rods) bore the names of the reagents. A great deal of comfort was derived from this very simple piece of apparatus.

The killing and staining was accomplished in two ways; either by killing first and staining afterwards, or by performing both operations simultane-

[4] See formulary of reagents at end of paper.

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ously. The killing fluids used were: a 10% aqueous solution of tannic acid, a 1% aqueous solution of copper sulphate, a 2% aqueous solution of osmic acid, a 4% aqueous solution of acetic acid, a 3% aqueous solution of mercuric chloride, a 1% aqueous solution of formaldehyde. The osmic acid and copper sulphate solutions seemed to be the best killers, killing the animals at once, and without apparent distortion. Neither did disintegration set in with such rapidity as was the case when some of the other killing reagents were employed. These killing reagents can be used in other strengths than those given here but these percentages seemed to give the best results.

The killing was done either with a large amount of the material in a watch crystal, or underneath the cover glass, and the staining was accomplished in the same way. Where the protozoa were extremely abundant, as they were usually in surface scums or infusions of decaying marine algae, the watch crystal "mass" staining or killing was found to be the most satisfactory, as well as the easiest and quickest. This method had also to commend it the fact that both the killing reagent and the stain could be most readily controlled. Several watch crystals full of material were placed side by side and very delicate gradations of color secured.

As has been previously stated, the intra vitam staining gave by far the best results. This was accomplished either under the cover glass, or in the watch crystals, following the methods noted above, after the gelatine had been added and the proper degree of viscosity secured.

Preparation of Cultures

In order that a large number of individuals of a given species may be available for examination, it is necessary to depend upon cultures. For convenience in designation, there have been recognized in this paper the following types: (1) natural cultures, that is, those in which large numbers of a species appear, in natural conditions in the field and without any artificial manipulation of the medium in which they occur, (2) indirect cultures, or those which result from merely collecting the material and allowing it to stand and to decompose in the laboratory, and (3) artificial cultures, or those which are prepared with a definite nutritive medium (determined by experimentation) and inoculated with the desired species.

There is little or no exercise of technique involved in securing either natural or indirect cultures. One soons learns to recognize good natural culture environments such as greenish duck ponds, for *Euglenae* of various species; boggy water supporting growths of *Sphagnum*, for *Prorodon niveus* and *armatus;* watering troughs with *Spirogyra* or other *Chlorophyceae*, for species of *Chilodon* and *Holophrya;* clear, cold waters for *Astasia*, etc.

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For indirect cultures one has merely to allow the collected water and vegetation to stand in the warmth and light of a south-exposed laboratory window, and make regular examinations day by day.

Where, however, but few individuals of a desired species occur, it becomes necessary to aid their propagation artificially. Results which gave earnest of better ones with further experimentation, were obtained by what is here termed artificial culturing. This was accomplished by segregating desired individuals, and then introducing into the jar of water in which they were placed some favorable nutritive substance. The methodology of preparing such cultures has been well enough developed at the present time, possibly, to make an account worth while, though many problems of detail still await solution.

For capturing individual protozoa under the microscope, there was devised what is here called an *isolation pipette*, shown in Fig. 4. A soft glass tube is drawn out to a hair-like degree of fineness at one end, and inserted into a thin walled rubber tube at the other. The opposite end of the rubber tube is tightly closed by means of a sealed glass tube. The hair-like point of the pipette is first dipped into clear water to allow capillary attraction to draw as much as it will up into the bore. The forefinger of the left hand is laid lightly upon the rubber tube near to its closed extremity, compressing it slightly and thereby driving out a small drop of the water from the tip of the glass pipette. The latter is now inserted with the right hand underneath the objective and into the uncovered drop on the slide. Release of the pressure of the left forefinger results in the withdrawal into the hairlike bore o the pipette a small quantity of water, the amount of which can be delicately regulated.

After the desired animal has been thus captured it is forced out into the water in the isolation jar, and there is added the proper nutritive substance. Thereafter the whole is set in a warm, light place to "ripen." It was found advisable, from the standpoint of ease of handling, to make the culture in small 3 or 4 cm. stender dishes. To inoculate such small cultures it sufficed, on several occasions, to introduce but a single individual. This, however, it must be confessed, was because we could secure no more, and the successes resultant from this meagre inoculation were regarded merely as fortunate accidents.[5]

Several inoculations, aggregating some half dozen, or preferably more, individuals are usually necessary. There was no certain way of determining, save after the anticipated development of the culture, whether the

[5] Single individuals can be removed by means of the isolation pipette, and introduced, for long-continued observations, into a device termed the *micro-aquarium* (See Hausman, L. A., The Vibratile Oral Membranes of *Glaucoma scintillans*, Ehr., Am. Nat., vol. 44, 1920, p. 427.

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animals had actually been introduced into the isolation jar and inoculation actually accomplished. Rather clumsy attempts, yet in several instances not unsuccessful ones, were made to make as certain as possible the incarceration of single individuals within the isolation jar by first ejecting the captured animal into a drop of clear water on a slide while under the microscope, noting the presence of the creature, and then washing it off carefully into the material in the isolation jar with a fine stream of clear water.

Tubes of different tip diameters were used, and it was noted that the most success attended the use of the finest of these which it was possible to use for a given species. It is well to give the tip a slight turn when drawing it out, as shown in the figure. This seemed to make it easier to manipulate under the microscope.

It was found practicably impossible to manipulate the pipette and to capture the protozoa under any power greater than that afforded by the use of the 10x eyepiece and the 16 mm. objective. To insure the best results the drop of water must be well flattened out, and first freed from annoying débris.

For maturing the cultures rapidly, and under conditions which could be regulated and tabulated for further referene, a culture oven, such as is illustrated in Figure 2 was devised. A large aquarium jar was equipped with perforated tin shelves hung by copper wires from the upper rim of the jar; heated with a carbon filament lamp, placed on a copper wire platform to prevent it coming into contact with the glass bottom of the jar, and covered with a cardboard cover bearing a thermometer. This had the advantage of furnishing to the stender dish cultures placed on the shelves, at once the requisite amounts of heat and light. The temperature of the interior of the oven was regulated by raising or lowering the cardboard cover, propping it up with little wooden blocks.

Samples were dessicated in this oven by removing the cover and allowing the cultures to remain until they had dried. In this way cultures of *Holophrya*, *Prorodon*, and *Loxophyllum* were kept and resuscitated at pleasure. This method of keeping material by dessication might be a useful one for class requirements. The dried material could be removed from the dishes and placed in labelled envelopes, and filed away in a card catalogue tray. More experimentation along this line might reveal the fact of its being possible to have on file any quantity of protozoa material which could be revivified at will for class room use!

Key to the Families and Subfamilies

I. Protozoa possessing, at some stage of the life cycle, locomotor appendages in the form of cilia, either single or fused into membranes. Macro and micro nucleus present. CLASS INFUSORIA

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II. Cilia present during the entire life cycle; buccal cavity and anal orifice normally present; contractile vacuole often connected with an excretory tube system.

SUBCLASS CILIATA

III. Cilia more or less alike in form and distribution over the entire body, having a tendency to lengthen (or in some cases to be present only) on the oral or aboral side. Buccal cilia usually a trifle longer than the others.

ORDER HOLOTRICHIDA

IV. Lacking undulating membranes about the buccal cavity, the latter being closed except during the ingestion of food.

SUBORDER GYMNOSTOMINA

A. Body outline usually oval or extended; neither oral nor aboral sides flattened

Family Enchelinidae

AA. Body outline sometimes oval or extended, more often, however, with oral side either flattened or concave.

B. Buccal cavity terminal or nearly so

Family Trachelinidae

BB. Buccal cavity not terminal

C. Gullet with pronounced curve

Family Enchelyidae

CC. Gullet without pronounced curve

D. Body entirely and evenly ciliated

Subfamily Nassulinae

DD. Cilia longer on, or confined to aboral side

Subfamily Chilodontidae

DDD. Cilia confined to oral side

Subfamily Erviliinae

KEY TO THE GENERA MENTIONED IN THIS PAPER

A. Body ovoid, ellipsoidal, or almost spherical

B. Body distinctly ovoid or spherical

C. Posterior spinous process present.....UROTRICHA

CC. Posterior spinous process not present

D. Possessing spiral band of longer cilia.....PERISPIRA

DD. Not possessing spiral band of longer cilia

E. Cilia restricted to one or two circles about the body

F. One midway circle of cilia present.....MESODINUM

FF. Two such circles present.....DIDINIUM

EE. Cilia not restricted to one or two circles about the body

F. Cilia restricted to one side of body.....TROCHILIA

FF. Cilia not restricted to one side of the body

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G. Buccal cavity anteriorly terminal
H. Nucleus long and curved.....ENCHELYODON
HH. Nucleus usually ovoidal.....HOLOPHRYA
GG. Buccal cavity not anteriorly terminal
H. With a short neck like, or lip like projection from anterior end.....TRACHELIUS
HH. Without such a projection.....NASSULA
BB. Body not ovoid or nearly spherical, but drawn out into the form of an elongated ellipsoid
C. Body armored with rectangular plates
D. Cilia arising from middle of rectangular plates..... COLEPS
DD. Cilia not arising from middle of plates.....TIARINA
CC. Body not armored with rectangular plates
D. Cilia restricted to ventral surface
E. Body about five times as long as, or longer than, greatest diameter.....LIONOTUS
EE. Body less than five times as long as its greatest diameter .....LIONOTOPSIS
DD. Cilia not restricted to the ventral surface
E. Body longitudinally furrowed.....PLAGIOPOGON
EE. Body not longitudinally furrowed.....PRORODON
AA. Body not ovoid, ellipsoid, or almost spherical
B. Body purse or flask shaped
C. With long flexible neck or proboscis
D. With circle of longer cilia about the anterior extremity of the proboscis.....TRACHELOCERCA
DD. Without such a circle of cilia.....LACRYMARIA
CC. With short neck
D. Neck obliquely truncated
E. Ciliation entire
F. Body capable of distortion at will.....ENCHELYS
FF. Body not capable of distortion.....SPHATHIDIUM
EE. Ciliation not entire.....PHASCOLODON
DD. Neck not obliquely truncated
E. Tentacular process arising from the buccal cavity in the anterior end of neck.....ILEONEMA
EE. Without such a tentacular process TRACHELOPHYLLUM
BB. Body not purse or flask shaped
C. Body ribbon or leaflike
D. Body very elongate
E. Anterior end rounded.....AMPHILEPTUS
EE. Anterior end not rounded.....FLEXIPHYLLUM

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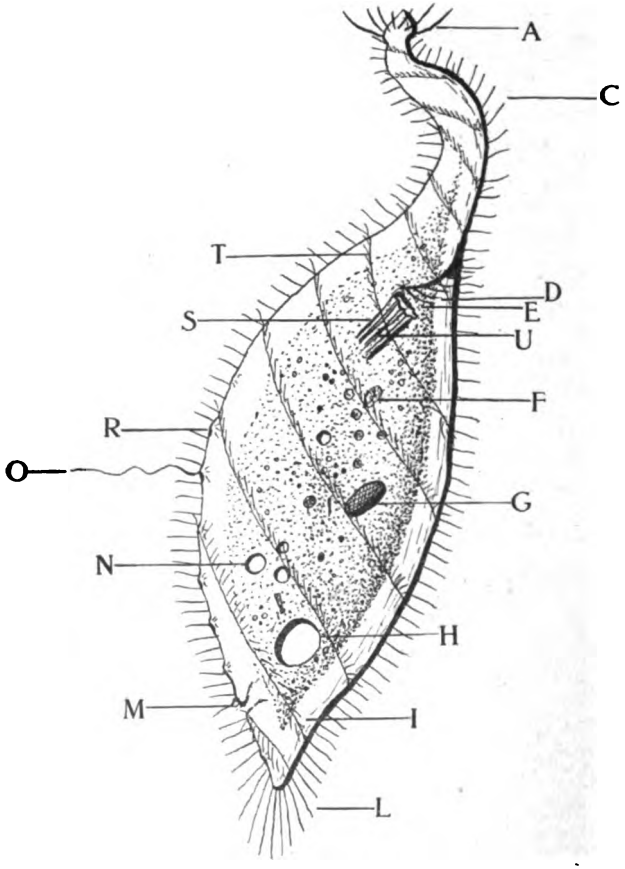

An ideal composite gymnostominan ciliate, to show the various anatomical divisions and organs of the body.

a. proboscis cilia
c. proboscis
d. buccal cilia
e. buccal cavity opening into pharynx
f. food vacuoles
g. nucleus
h. principal contractile vacuole
i. hyaline border of body
l. caudal cilia
m. anus
n. smaller contractile vacuole
o. discharged trichocyst
r. undischarged trichocyst
s. pharynx
t. cila band
u. pharyngeal rods

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DD. Body not very elongate
E. With anterior border crenulate.....LOXODES
EE. Without crenulations on the anterior border
F. Neck elongated and constricted.....DILEPTUS
FF. Neck not elongated and constricted..LOXOPHYLLUM
CC. Body not leaflike; usually kidney or bean shaped, or nearly so
D. Pharynx long and curved.....TILLINA
DD. Pharynx short and straight.....CHILODON

Observations on the Recorded Species

Genus *Holophrya*

This genus, a very common one, and widely distributed, possesses either an enormous number of distinct species, or a much smaller number, many of which are very variable in size and form, and often, indeed, in coloration. From our limited observations we are inclined to take the latter view. Little attempt has heretofore been made to accord all of these diverse forms specific names.

The species here called Sp. 1 and 6 were commonly found in brackish tidal estuary water about a mile from Long Island Sound among detached, floating *Fucus* and green Sea Lettuce, the first day after having been brought into the laboratory. All of the individuals of these species observed (and there must have been hundreds seen) varied but little away from an average. Later on, however, considerable variation occurred as the numbers became greater in the slowly putrescing material. It is difficult to say whether this was due, however, to an increasing variation among the individual members of the species, or whether new species were making their appearance.

Species 5, 6, and 7 were present in enormous numbers in a four days old infusion of fresh cabbage leaves, and in an infusion of dried corn leaves of the same age.

Species 2, 3, and 4 were met with occasionally in almost all samples, both marine and fresh water, particularly in those from ponds.

The globularity of body, and the small anterior or anterior-lateral buccal cavity, together with the uniform length and distribution of the cilia, appear to be constant characteristics of the members of the genus. Among such small, globular forms, mutilated individuals seemed not to be very common. We are of the opinion that figures of very irregular forms assigned to this genus were made from such mutilated individuals.

Holophrya sp. 1 to 7 shown in Figs. 5-11.

Genus *Urotricha*

Members of *Urotricha* may easily be recognized by the presence of the caudal spine or seta, and by their curious habit of swimming slowly and

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evenly and then suddenly jerking ahead, or to the right or left, as though shot by a spring, a motion resulting from a quick lateral snap of the rigid caudal seta.

Kent has observed that the walls of the pharynx are surprisingly elastic, and that this enables the creatures often to take in food, the bulk of which may equal their own bodies!

If the posterior spine is not easily seen, staining makes it easily visible. *Urotricha farcta* and *platystoma*, Figs. 12 and 13.

Genus *Perispira*

The *Perispira ovum* (Fig. 14), of Stein, which we have recorded from stagnant pond waters, may be the same as the *Holophrya ovum*, of Ehrenberg. If this be so then it is an aberrant form of *Holophrya*, for it possesses a spiral band of longer cilia characteristic of *Perispira*. Since even ciliation is characteristic of *Holophyra*, this form had best be placed in *Perispira*.

Genus *Enchelys*

This genus is hardly distinguishable from *Holophrya*, the presence of the laterally opening buccal cavity of *Enchelys* being apparently the sole point of difference. And when the members of the latter assume a globular form, which they do with apparent volition, the buccal cavity becomes almost exactly anteriorly terminal, much like that of many species of *Holophrya*. This assumption of globularity occurs, often, when the animal is gorged with food granules. The young, soon after division has been completed, also take on a globular body.

The smaller species figured is regarded as the *Enchelys farcimen* (Fig. 16) of Ehrenberg. *E. pupa* (Fig. 15) was met with several times in pond water.

Genus *Enchelyodon*

The ovate-elongate body and the terminal buccal cavity, together with the large size, should serve to distinguish *Enchelyodon farctus* (Fig. 17) from forms in the genera *Prorodon* and *Enchelys*. Note that the cilia are very short. We were unable to see them in the unstained animal. This form was rarely found in the waters of bogs, ponds, slowly moving streams, etc.

Genus *Spathidium*

The chief difference between this genus and *Enchelys*, from which it is separated only with difficulty, appears to be the possession of a longer pharynx, usually furnished with pharyngeal rods. The latter, however, are not easily visible.

The species figured, which seems to be *Spathidium spathula* (Fig. 18), was found in pond and slow stream waters.

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Genus *Prorodon*

Both *Prorodon armatus* (Fig. 19) and *P. ovum* (Fig. 20) were rarely found in pond waters. The buccal cavities of both are distinct, and the prominent pharyngeal rods of the latter were very good as an identification characteristic.

Genus *Lacrymaria*

Lacrymaria olor (Fig. 21) is a common form in infusions of leaves both of deciduous tress and of aquatic plants. Like *Trachelocerca olor* (Fig. 22) it often lies with its lenticular body concealed among a mass of débris and shoots forth its long serpentine neck in all directions. Whether this is a deliberately willed concealment for the purpose of protection, or for the advantage which it secures for the seizure of prey is uncertain. I have not seen this habit mentioned elsewhere, and yet I found it to be a very common one among the many individuals observed.

Its size is extremely variable, but the constant body form offers a ready means for identification.

Genus *Trachelocerca*

To be distinguished from *Lacrymaria* chiefly by the smaller size of its members. *Trachelocerca olor* (Fig. 22) and *Lacrymaria olor* are almost identical in habits. The movements of the smaller form are, however, the more rapid. *Tracheolocerca olor* is found commonly among the smaller aquatic vegetation in small quiet pools and coves.

Trachelocerca phoenicopterus (Fig. 23) is a marine species occurring among algae along the shore, as well as in putrifying infusions. Its length seems to be very variable.

Genus *Ileonema*

Ileonema dispar (Fig. 24) occurs among *Spirogyra*, *Zygnemea*, *Oscillatoria*, and can probably be found among any of the fresh water filamentous algae. It is not a common form, and usually disappeared soon from fresh material.

The cilia are sparse and apparently weak.

Genus *Plagiopogon*

Plagiopogon coleps (Fig. 25) which we figure from Kent, we believe to have found in salt water among decaying *Fucus* and other algae. It closely resembles *Coleps hirtus* (Fig. 26) though the longitudal furrows of the body and the absence of armor plates are apparent under high powers. It seems to be a species of fairly constant form and size.

Genus *Coleps*

Coleps hirtus (Fig. 26) is a very common form of ciliate, the commonest of its genus, among decaying vegetation and in old infusions, and can be

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readily identified from its size and armored body. In swimming it twirls rapidly on its longitudinal axis and pursues a rapid, wavering reckless course. It is an exceedingly voracious species and appears to feed on both animal and vegetable tissue, and the bacteria which are disintegrating them.

Genus *Tiarina*

Tiarina fusus (Fig. 27), a marine form from among decaying algae, resembles *Coleps* in structure very closely. The form of the body is, however, different. The form which we figure we take to be *Tiarina fusus*, (Fig. 27) which is apparently the same as the *Coleps fusus* of Claparède and Lachmann.

Genus *Didinium*[6]

Didinium nasutum (Fig. 28), not an uncommon form in decaying and fresh aquatic vegetation, is one of the largest of the ciliates. Its two zones of cilia offer an easy character for identification. This form appears freely where an adequate supply of smaller ciliates appear, for it is upon these that it feeds. The habits of this species have been exhaustively studied by S. O. Mast (22) and recorded in one of the most interesting of the recent papers on protozoan habits.

The natatory movements of this species are much like those of *Urotricha*, namely a slow gliding progression interrupted frequently by spasmodic jerks.

Genus *Mesodinium*

Mesodinium cinctum (Fig. 30) is not an uncommon form in salt water and when swimming rapidly looks very much like a minute replica of *Didinium nasutum*. The constricted median line and the single zone of median cilia make it easy to identify when at rest.

The smaller *Mesodinium* (Fig. 29), which is found rather rarely associated with the preceding species, I consider to be the *Mesodinium pulex* of Claparède and Lachmann.

Genus *Tillina*[7]

Tillina magna (Fig. 31) were found frequently in a ten day's old infusion of dried corn leaves associated with various species of *Holophrya*, *Chilodon*, and *Colpoda*. Its distinguishing characteristics are the irregular, asymmetrical body and the curved, ciliated pharynx.

Genus *Amphileptus*

Fig. 32 I have called provisionally, *Amphileptus gutta*. It seems to occur in both marine and fresh water infusions. They bear either many smaller contractile vacuoles distributed over the posterior two-thirds of the body,

[6] See Mast, S. O., The Reactions of *Didinium nasutum*, etc., Biol. Bull., vol. 16., 1908, p. 91.

[7] See Gregory, L. H. Observations on the Life History of *Tillina magna*, Jour. Exp. Zool., vol. 6, 1909.

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or occasionally, yet not so frequently, one single large vacuole, situated in the posterior half or posterior end, or slightly to one side. Because of a lack of very definite characteristics forms like this are difficult to place with certainty.

Genus *Lionotus*

Members of this genus are among the most graceful ciliates. Viewed from above, the apparently slender neck is seen to be broad and leaflike. Figures of these species should, therefore, indicate this and not lead to the impression that the neck is of the same type as that possessed by *Lacrymaria* or *Trachelocerca*.

Lionotus wrzesniowski (Fig. 36) is a large form, found in pond waters amid living and dead aquatic vegetation, where often occurs, also, *Lionotus fasciola* (Fig. 34). The latter species is also found in salt water with *Fucus* or other marine algae. A similar form, entirely restricted to fresh water, is *Lionotus pleurosigma* (Fig. 35). This species can be distinguished from *fasciola* only by its clear, deep, hyaline border.

The smallest of the species figured (Fig. 33) was found in brackish water in a tidal estuary among detached, floating marine algae.

Genus *Lionotopsis*

Fig. 37 is, perhaps, the *Lionotopsis anser* of Conn, drawn from but a few poorly defined individuals found in pond water. The position of the buccal cavity could not be determined.

Genus *Loxophyllum*

Members of this genus can usually be recognized by the gracefully flexible way in which they glide about over and through débris or wrap their pliant and leaflike bodies about it. The ease with which the curved anterior portion of the body is used for the examination of possible food substances reminds one of the sensitive exploratory gropings of the tip of an elephant's trunk. The deep, clear, hyaline border possessed by all the Loxophylla is constantly characteristic.

Loxophyllum setigerum (Fig. 39) and *rostratum* (Fig. 40) were found quite abundantly in brackish water. The latter appeared in great abundance in an eight days old infusion of green Sea Lettuce and *Fucus* in salt water.

Loxophyllum sp. 1 occurred in fresh water among aquatic vegetation (Fig. 38).

Genus *Trachelophyllum*

Fig. 41 has been called *Trachelophyllum tachyblastum*, from a single specimen found in pond water.

Genus *Flexiphyllum*

Flexiphyllum elongatum (Fig. 42) is frequently met with in pond water among growing vegetation. Its motion is a graceful and sinuous gliding

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and it makes rapid progress through the water. We have found that it prefers to move concealed amid débris.

Genus *Trachelius*

Trachelius ovum (Fig. 43) possibly the most common species, can be distinguished by its large size, its curious little neck, and its deliberate motions. The buccal cavity and gullet are quite prominent in most individuals. The size and shape of the neck is apparently subject to considerable variation. Within the body the number of contractile vacuoles is normally very large.

Genus *Dileptus*

Dileptus gigas (Fig. 44), fairly common form, is of unusual variability in size and shape. It is entirely carnivorous and possessed of a voracious appetite. The prey is stung and rendered helpless by the discharge of the trichocysts located along the border of the long neck like process, and if too large to be swept into the buccal cavity by the lashings of the buccal cilia is forced in by the writhings of the neck. The body often rotates on its longitudinal axis during progression through the water.

Individuals have been reported which measured 800 μ.

Genus *Loxodes*

Loxodes rostratum (Fig. 45) was found only rarely in pond water among fresh and decaying vegetation. It is reported to occur also commonly, in infusions.

Genus *Nassula*

This is a beautiful genus, its members being symmetrically ovoid, and many of them iridescent. *Nassula microstoma* (Fig. 48) is a very pretty species. It is usually brownish or yellowish, the color depending upon its contained food. Under strong light, as it revolves through the water, it scintillates brightly, reminding one of a small, ovoid, minutely faceted epidote. This was a very common species in brackish tidal estuary water.

Nasulla ornata (Fig. 46 and *Sp. 1* (Fig. 47) were found in pond waters among fresh and decaying vegetation.

Genus *Chilodon*

Chilodon, much like *Holophrya*, is a genus containing a great number of species of considerable variability of form and size. Of all the species which vary in this way among themselves, *Chilodon cucullulus* (Fig. 52), the commonest, is the most flagrantly disregardful of maintaining its proper dimensions and contour! In the same infusion we have found no less than a dozen differently shaped and sized specimens! Calkins says of this species that it is "extremely variable . . . and has received so many different names that it hardly pays to enumerate them all." It is "one of the most common and widely spread ciliates known."

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This species appeared, in a remarkably pure and rich culture, in a five days old infusion of dried flaky deposit from the sides of an old wooden watering trough. It occurred also abundantly in fresh and putrid sea water.

Chilodon megalotrocha (Fig. 49) and *caudatus* (Fig. 51) are sometimes found associated with cucullulus. *Chilodon vorax* (Fig. 50) is much less common.

Genus *Phascolodon*

Phascolodon vorticella (Fig. 53) is a freshwater form from swamps, rather rare. It is a beautiful form, graceful and deliberate in its movements. The body plasm is clear and crystalline.

Genus *Scaphidiodon*

Scaphidiodon sp. 1 (Fig. 54) is perhaps the form shown in Fig. 54. This occurs in sea water containing putrid animal and plant tissues.

Genus *Trochilia*

Trochilia sigmoides (Fig. 55) is another marine form, very striking and beautiful. It is found rarely in clear salt water with living algae, and can be easily recognized by the oblique banding on the body and the meagre number of cilia restricted to one side of the body. Near the small, anterior end of the body is located the buccal cavity, apparently not followed by a definite pharynx, but surrounded by cilia a trifle longer than the rest. It swims slowly, rotating.

FORMULARY OF REAGENTS FOUND USEFUL IN QUIETING, KILLING AND STAINING

I. Quieting Solutions

1. Gelatine solution—Water.....5 oz.
   Gelatine.....1 oz.

   Heat slowly until gelatine is dissolved; then allow to cool and congeal to the desired viscosity.
2. Chlorotone—a 1 per cent aqueous solution

II. Killing Reagents

1. 10 per cent aqueous solution tannic acid
2. 2 per cent aqueous solution osmic acid—Invert slide with drop of water containing the protozoa over a bottle of the solution, uncorked. The fumes kill almost instantly.
3. 3 per cent aqueous solution acetic acid
4. 1 per cent aqueous solution copper sulphate
5. 2 per cent aqueous solution chromic acid

III. Staining Reagents (These can be made up as saturated solutions in either water or 95 per cent alcohol, and diluted to the desired

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depths of color. When used in aqueous solutions, very dilute, they make good intra vitam stains.)

1. methyl blue
2. methyl green
3. Lichtgrün
4. Bismark brown
5. Safranin
6. gentian violet
7. iodine, with potassium iodide (a killing stain, either with water or alcohol).

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EXPLANATION OF PLATE

Fig. 1. Stain or reagent set holder.

Fig. 2. Culture oven *a*, switch, *c*, cardboard cover, *d*, tin shelf, *e*, lamp rack, *f*, copper wire for suspending shelves, *g*, thermometer.

Fig. 3. Strainer for concentrating samples.

Fig. 4. Isolation pipette

Fig. 5. Holophrya sp. 1, 15–25μ

Fig. 6. Holophrya sp. 2, 15–25μ

Fig. 7. Holophrya sp. 3, 15–25μ

Fig. 8. Holophrya sp. 4, 15–25μ

Fig. 9. Holophrya sp. 5, 45–55μ

Fig. 10. Holophrya sp. 6, 30–35μ

Fig. 11. Holophrya sp. 7, 40–45μ.

Fig. 12. Urotricha farcta, 15–25μ

Fig. 13. Urotricha platystoma, 35–45μ

Fig. 14. Perispira ovum, 80–100μ

Fig. 15. Enchelys pupa, 80–100μ

Fig. 16. Enchelys farcimen, 25–50μ

Fig. 17. Enchelyodon farctus, 175–225μ

Fig. 18. Spathidium spathula, 60–80μ

Fig. 19. Prorodon armatus, 25–30μ

Fig. 20. (See Plate II)

Fig. 21. (See Plate II)

Fig. 22. Trachelocerca olor, 320–380μ

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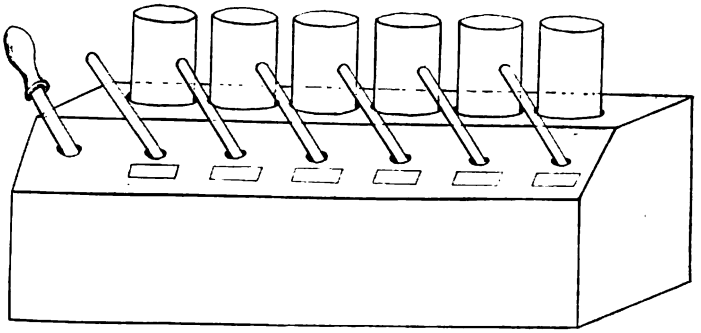

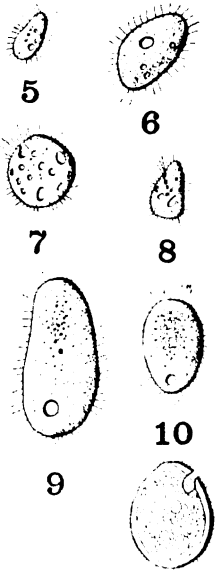

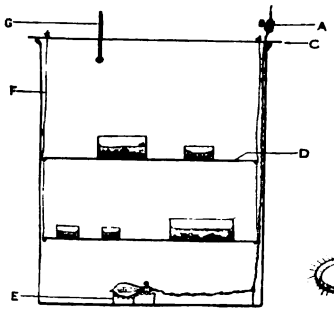

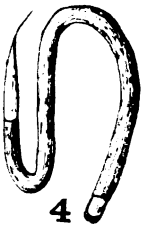

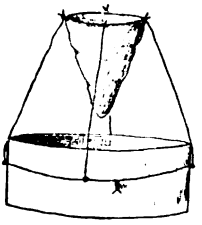

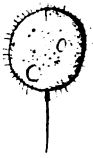

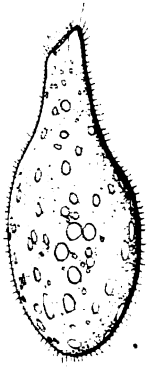

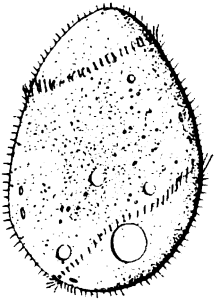

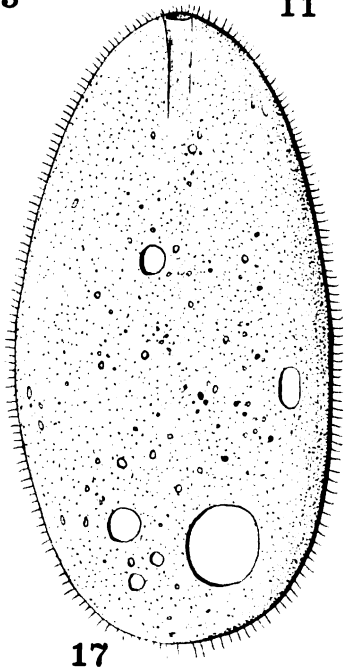

PLATE V

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EXPLANATION OF PLATE

Fig. 20. Prorodon ovum, 100–130μ.

Fig. 21. Lacrymaria olor, 320–380μ.

Fig. 23. Trachelocerca phoenicopterus, 450–1000μ.

Fig. 24. Ileonema dispar, 115–125μ.

Fig. 25. Plagiopogon coleps, 75–90μ.

Fig. 26. Coleps hirtus, 45–55μ.

Fig. 27. Tiarina fusus, 75–80μ.

Fig. 28. Didinium nasutum, 850–1000μ (the only species not drawn to scale. If represented in its relative proportions, it would be more than twice and a half as large as Trachelius ovum, Fig. 43, Plate IV).

Fig. 29. Mesodinium pulex, 10–20μ.

Fig. 30. Mesodinium cinctum, 30–45μ.

Fig. 31. (See Plate III).

Fig. 32. Amphileptus gutta, 40–60μ.

Fig. 33. Lionotus sp. 1, 25–35μ.

Fig. 34. Lionotus fasciola, 75–125μ.

Fig. 35. Lionotus pleurosigma, 110–125μ.

Fig. 36. Lionotus wrzesniowski, 175–200μ.

Fig. 37. Lionotopsis anser, 75–100μ.

Fig. 38. Loxophyllum, sp. 1, 45–50μ.

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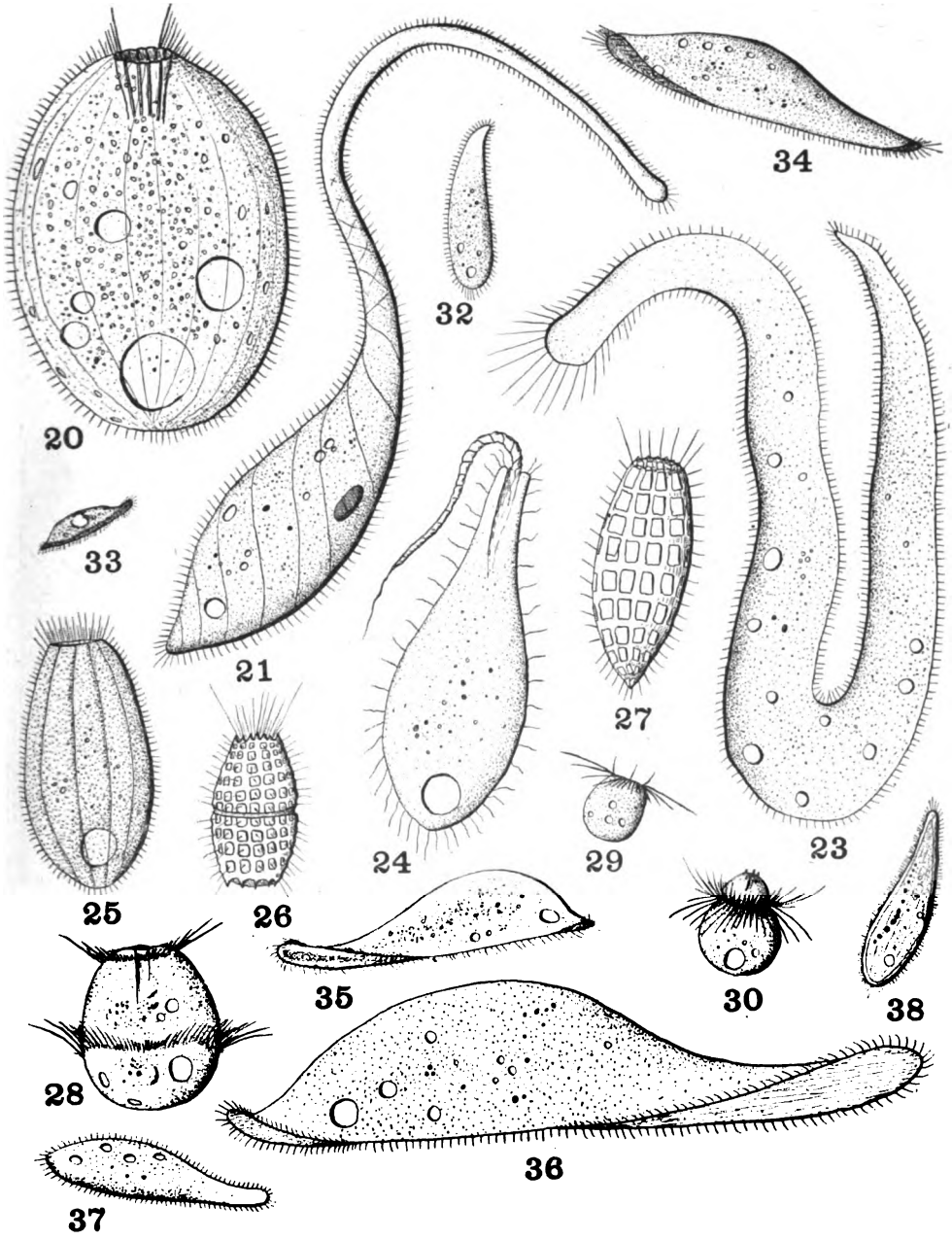

PLATE VI

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EXPLANATION OF PLATE

Fig. 31. Tillina magna, 195–225μ.
Fig. 39. Loxophyllum setigerum, 100–125μ
Fig. 40. Loxophyllum rostratum, 125–150μ
Fig. 41. Trachelophyllum tachyblastum, 120–150μ
Fig. 42. Flexiphyllum elongatum, 200–300μ
Fig. 43. (See Plate IV)
Fig. 44. (See Plate IV)
Fig. 45. Loxodes rostratum, 250–350μ
Fig. 46. Nassula ornata, 200–400μ
Fig. 47. Nassula sp. 1, 30–35μ
Fig. 48. Nassula microstoma, 50–60μ
Fig. 49. Chilodon megalotrocha, 40–50μ
Fig. 50. Chilodon vorax, 50–70μ
Fig. 51. Chilodon caudatus, 35–50μ

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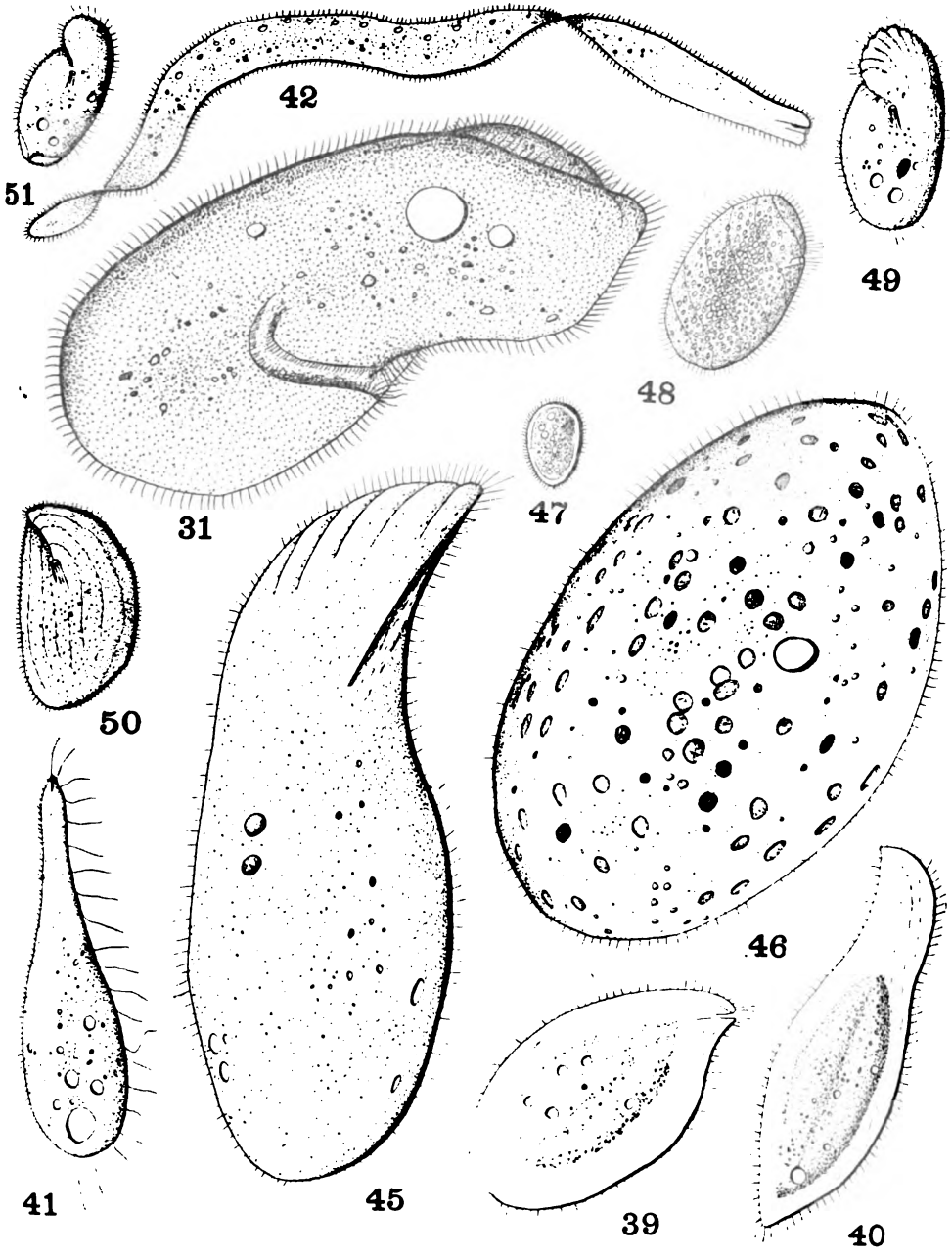

PLATE VII

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EXPLANATION OF PLATE

Fig. 43. Trachelius ovum, 280–350μ
Fig. 44. Dileptus gigas, 450–800μ
Fig. 52. Chilodon cucullulus, 125–225μ
Fig. 53. Phascolodon vorticella, 40–70μ
Fig. 54. Scaphidiodon sp. 1, 25–35μ
Fig. 55. Trochilia sigmoides, 30–40μ

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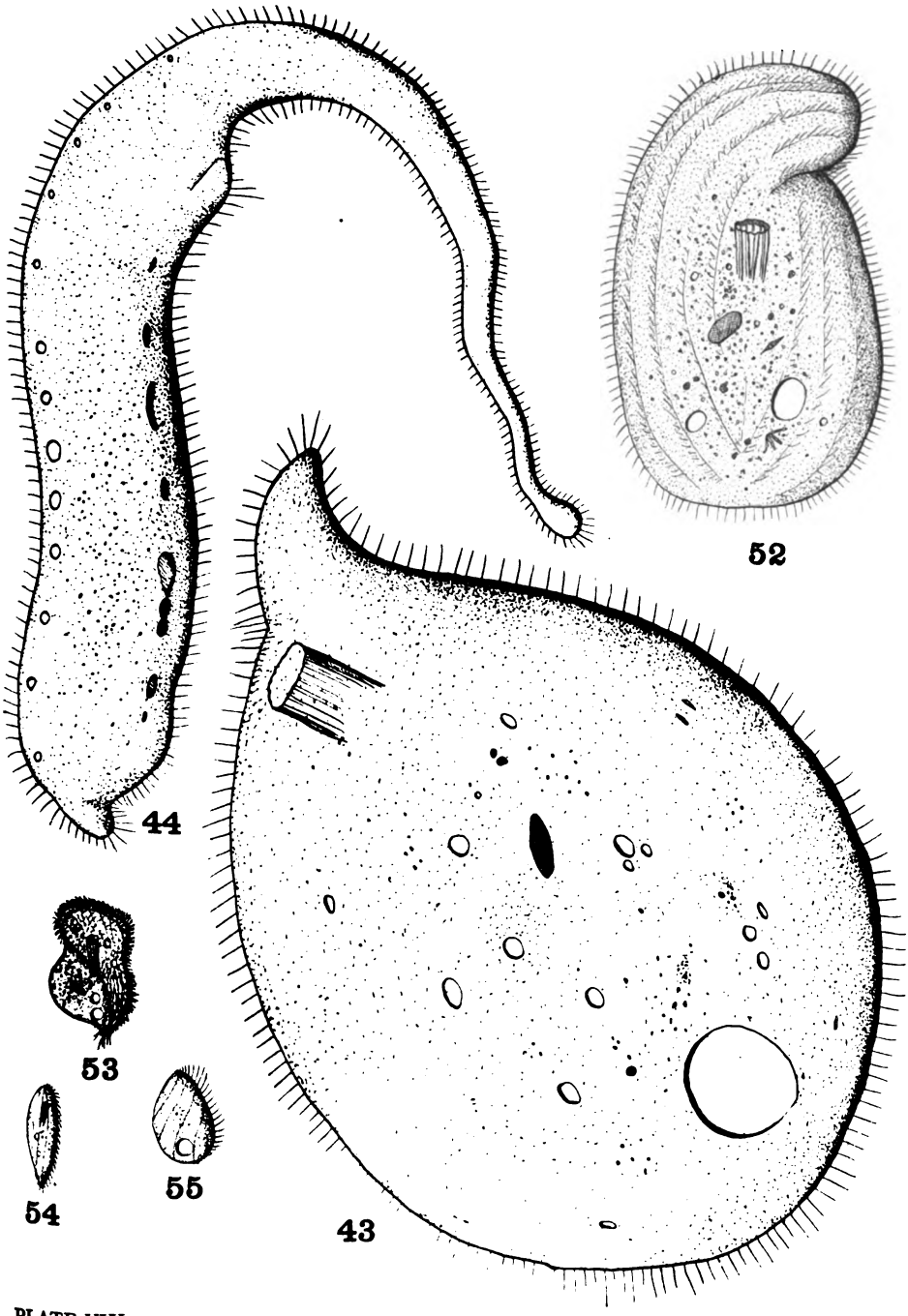

PLATE VIII

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COPPER: ITS OCCURRENCE AND RÔLE IN INSECTS AND OTHER ANIMALS[1]

By

Richard A. Muttkowski, Ph.D.
University of Idaho, Moscow, Idaho

1. General.
2. Respiratory Proteins in Insects; the Copper Nucleus.
3. Copper in Other Animals.
4. The Sources of Copper: Soil, Water, Plants.
5. Discussion.
6. Summary.
7. Bibliography.

I. General

The present paper arose from certain experiments on the respiration of insects, particularly on the gases of the blood, and its rôle as a respiratory factor.

As at present understood, respiration in insects proceeds by the tracheal method: Atmospheric air is led directly to the cells by the tracheae, while the blood acts primarily in the transportation of food and metabolic products. This is modified in aquatic stages, in some of which the oxygen in solution in the water is absorbed by means of tracheal gills. In certain *secondarily* aquatic insects,—that is, insects originally aquatic which became terrestrial in habit, but which in some stages again have sought the water,—there are found structures which fundamentally are of the gill type. Here a thin membrane separates the blood from the water; the blood takes up oxygen through this membrane and distributes it directly to the tissues, or indirectly by yielding it to the gaseous supply in the tracheae. Such structures are the gill filaments and gill pouches of Trichoptera larvae and aquatic caterpillars, the caudal gill pouches of Chironomid larvae, Culicid larvae, Simulium larvae, etc.

Considered from both the physiological and morphological standpoint, these structures are meaningless unless a respiratory protein is postulated in the insect blood to fix the transfusing oxygen. Without such a protein, there could exist only an oxygen balance between the fluids divided by the animal membrane,—between the oxygen in solution in the blood serum and that in solution in the water. But since the available dissolved oxygen decreases with the rising temperature of the water, and the temperature of the insect rises with that of its environment, the oxygen supply of the water becomes impoverished, and with it the amount in solution in the blood,— that is, if the blood lacked a respiratory protein.

[1] (Contribution from the Zoological Laboratory of the University of Idaho, Moscow, Idaho.)

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Furthermore, many insects are found in places where the available oxygen is nearly entirely used up in the decomposition and oxidation of organic waste. Indeed, a number of species are known which live, grow, and transform under anaerobic conditions (Juday 1909, Muttkowski, 1918).

Now, a respiratory protein is known for a few insect species, among them some of the anaerobes just referred to, especially the so-called "blood-worms" or Chironomid larvae. This pigment has been identified as hemoglobin, dissolved in the blood plasma, and not included in the corpuscles. As far as known to the writer, it is restricted among insects to the "blood-worm" type of Chironomid larvae. The important point in connection with the hemoglobin of these larvae is this: It is confined to a few species, but not all of these species live under anaerobic conditions; nor do all anaerobic Chironomids contain hemoglobin. One is forced to the conclusion that among these latter the hemoglobin is replaced by some other respiratory pigment. Hemocyanin has been suggested by the writer (1920), altho it had not been demonstrated for a single insect species.

It is known from the study of vertebrate blood that hemoglobin forms oxyhemoglobin with oxygen and carbohemoglobin with carbon dioxide. From its identity with vertebrate hemoglobin it can be supposed that the activity of the hemoglobin as found in the Chironomid "blood-worms" is similarly two-fold,—that it transports both oxygen and carbon dioxide. For the rest of insects it was shown by the writer that both oxygen and carbon dioxide are present in the blood (account published elsewhere). Hence it is logical to assume that, similar to Chironomid "blood-worms," there is a definite carrier which fixes both oxygen and carbon dioxide.

The following recounts a series of experiments undertaken in an attempt to prove or disprove the foregoing assumption. The experiments were performed during the spring, summer and fall of 1920, altho some earlier observations made in the course of the past ten years are included.

II. Respiratory Proteins in Insects; the Copper Nucleus

In its development the problem presented several distinct phases: (1) Aside from the few insects possessing hemoglobin, is a respiratory protein available at least in those aquatic insects provided with blood gills? (2) If such a protein can be demonstrated, what is its nature? (3) If available, is it confined to aquatic stages or is its distribution universal among insects?

The presence of hemoglobin in Chironomid larvae (blood-worm type) is easily verified. For the blood responds to the various oxidation (Guiac, O-tolidin, and Benzidine reactions) and crystallization tests (Hemin) that have been elaborated for the recognition and study of hemoglobin in vertebrate blood. Except for the hemin test, none of these is conclusive

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as far as general differentiation between Invertebrate and Vertebrate blood is concerned. The hemin test alone indicates positively the presence of hemoglobin *as such* in Chironomid "blood-worms," or any other animal. Yet in a number of tests made for hemoglobin in the colorless blood of species like Anax, Aeshna, Dytiscus, and others, isolated crystals other than Sodium chloride were found which resembled the familiar prisms of hemin.

The oxidation tests are conclusive only in so far as they reveal the presence of blood, specifically the respiratory protein. They do not indicate the identity of this pigment. For it is noteworthy that the blood of crayfish as well as that of all insects reacts with Guaiac, and Benzidine, and produces color changes identical with those produced by vertebrate blood (See table I). Note that these oxidations are produced by blood which shows little or no trace of hemoglobin. Furthermore, as with vertebrate blood, boiling the test substance does not stop the reaction.

TABLE I. REACTIONS OF INSECT BLOOD TO HEMOGLOBIN TESTS

Name of Species	Guiac Test (Oxidation)		Benzidine Test (Oxidation)		Hemin Test-Crystals (Nippe's Reagent)	
	Number	Result	Number	Result	Number	Result
Aeshna larva.....	8	pos.	3	pos.	5	4 neg., 1 trace
Anax, yg. larvae....	2	"	1	"		
Enallagma larvae...	2	"	1	"		
Mayfly nymphs....	1	"				
Belostoma.....	3	"	1	"	7	5 neg., 2 trace
Chironomus larvae	6	"	3	"	12	12 pos.
Dytiscus larvae....	2	"			6	5 neg., 1 trace
Dytiscus adults....	2	"	1	"	7	5 neg., 2 trace
Controls						
Cambarus blood...	6	"	3	"	5	5 neg.
Blank, with $FeCl_2$..	5	"	3	"		
Blank, with $CuSO$	5	"	3	"		

In hemoglobin the iron is the active agent in the oxidation, in the hemocyanin of the crayfish it is the copper. That such is really the case was readily shown by the introduction of a crystal of ferric chloride or copper sulphate into some of the blank control tests. Such "salted" controls reacted positively, before and after boiling.

The tests described proved two things: (1) The oxidation tests for hemoglobin do not serve to differentiate between this and other respiratory proteins, or between the blood of Vertebrates and Invertebrates. (2) They proved the presence of a respiratory protein in insects.

Two possibilities at once presented themselves,—that the carrier in question could be either hemoglobin, or hemocyanin; or both, as in some

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mollusks. It was definitely ascertained, however, that in only a very few insects could hemoglobin be the respiratory protein. In most insects, if present at all, it was found only in infinitesimal quantities, and therefore negligible as a respiratory factor. This left the alternative of hemocyanin. This respiratory protein has been reported for a number of higher Crustacea and some Arachnida (Scorpion, Limulus). It is also widely known among the Mollusca, and there is no valid reason to assume that it might not be present in other groups of animals, including the insects.

To ascertain if this is the case, both direct and indirect methods were resorted to in this study. Unfortunately, no direct method for the recognition of hemocyanin is known such as the hemin test for hemoglobin. A large number of experiments were attempted to find such a reagent, but all were unsuccessful. Hence an indirect method was adopted.

In hemocyanin copper forms the nucleus of the respiratory compound. If the presence of copper could be shown in insect blood in amounts comparable to the copper content of equal quantities of crayfish blood,—then it would be logical to assume that this copper forms the nucleus of a respiratory protein similar to that of crayfish. Since, as already related, various tests indicated the presence of extremely minute quantities of hemoglobin, and since hemocyanin responded positively to the various hemoglobin tests, the latter were useless for differentiation between an iron and a copper compound. It therefore became necessary to separate the two, and to test separately for copper. This, of course, could be accomplished only after incineration of the tissues.

Among larger insects the blood and entire specimens, in small insects only whole specimens, were incinerated in the course of this study. The incinerations were begun in June 1920 and continued thru the summer and fall, as material became available. The usual analytical methods were followed: the ash was dissolved in hot dilute hydrochloric acid, a portion tested for iron, while the remainder was treated with excess of ammonia, precipitating the iron and dissolving the copper. The solution was then filtered, the filtrate concentrated by slow heating, acidulated with acetic acid, and tested for copper. Where the amount of ash was very small, the residue was redissolved and reprecipitated several times in order to obtain all the copper present.

As expected, iron showed heavily in all the incinerations, as it is universally present in animal cells. The thiocyanates were the chief reagents used in testing for this substance. Only qualitative tests were performed on copper, with notation of the approximate intensity of the reaction as compared with the control substance, namely crayfish blood. No exact quantitative estimates were possible, as the amounts dealt with

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were microscopic. The reagents employed were Potassium ferrocyanide, Ammonium mercuric sulphocyanate, especially after the test drop had been inoculated with zinc salts (acetate or sulphate) or Caesium and Rubidium chloride; and finally, the Lead acetate—Potassium nitrite method for the formation of the triple nitrite Lead-Copper-Potassium. The ammonia so generally employed as a test for copper was not sufficiently delicate. It is sensitive only to about 1:2000, and the copper obtained in the few milligrams of ash was insufficient to react with it. The other reagents mentioned are sensitive to copper in dilutions up to 1:50000 and over, sufficiently so to give definite reactions.

The incinerations covered practically every order of insects (see Table II). The material incinerated was collected by the writer from Paradise Creek, two or three ponds, and the fields in the immediate vicinity of the university at Moscow, Idaho; except no. 45, Sialis larvae, which were obtained from Lake Mendota, Madison, Wis., and kindly sent me by Prof. Chancey Juday, of the Wisconsin Geological and Natural History Survey.

TABLE II. COPPER IN INSECTS

Name	Stage	Tissue	No. of Incinerations	Result Cu	Remarks
Coleoptera					
1. Dytiscus	larva	blood	2	pos	Equal to Cambarus
2. "	"	whole	2	pos.	slightly less
3. "	adult	blood	5	pos.	nearly equal
4. "	"	whole	4	pos.	slightly less
5. Gyrinus	adult	whole	1	pos.	less
6. Harpalus	adult	whole	1	pos.	less
7. Leptinotarsa	"	"	1	pos.	less than C.
Hymenoptera					
8. Apis mellifica	adult	whole	2	pos.	less than C.
9. Bombus sp.	"	"	1	pos.	less
10. Polistes	"	"	2	pos.	nearly equal
11. Formica	"	"	1	pos.	less
Lepidoptera					
12. Pieris rapae	larva	blood	1	pos.	about equal
13. " "	"	whole	1	pos.	about equal
14. " "	adult	whole	2	pos.	less
15. Noctuid moths	adult	whole	2	pos.	less
Diptera					
16. Musca domestica	larva	whole	2	pos.	slightly more
17. " "	adult	whole	2	pos.	about equal
18. Stomoxys	adults	"	2	pos.	less
19. Tachinid flies	"	"	1	pos.	less

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TABLE II. COPPER IN INSECTS (*Continued*)

Name	Stage	Tissue	No. of Incinerations	Result Cu.	Remarks
Hemiptera					
20. Belostoma	yg. nymphs	blood	3	pos.	slightly less
21. "	yg. nymphs	whole	3	pos.	slightly less
22. "	adults	blood	6	pos.	about equal
23. "	"	whole	5	pos.	slightly less
24. Ranatra	"	"	1	pos.	" "
25. Gerris sp.	"	"	1	pos.	" "
26. Notonecta sp.	"	"	3	pos.	" "
27. Corixa sp.	"	"	2	pos.	" "
28. Aphis sp.	mixed	"	1	pos.	" "
Odonata					
29. Anax & Aeshna	yg. larvae	blood	2	pos.	less
30. Anax & Aeshna	yg. larvae	whole	2	pos.	less
31. Anax	larvae	blood	1	pos.	nearly equal
32. "	"	whole	1	pos.	less
33. Aeshna	"	blood	8	pos.	nearly equal
34. "	"	whole	7	pos.	" "
35. Sympetrum	"	blood	2	pos.	less
36. "	"	whole	2	pos.	"
37. Libellula	yg. larvae	whole	1	pos.	"
38. "	old larvae	whole	3	pos.	slightly less
39. Enallagma	yg. larvae	whole	1	pos.	less
40. "	late larva	"	3	pos.	about equal
41. "	adult	whole	2	pos.	less
Ephemeroidea					
42. Several spp.	nymphs	whole	1	pos.	slightly less
Trichoptera					
43. Several spp.	larvae	whole	1	pos.	nearly equal
Neuroptera					
44. Myrmeleon	adults	whole	3	pos.	equal or stronger
Megaloptera					
45. Sialis infumata	larvae	whole	1	pos.	nearly equal
Isoptera					
46. Termes sp.	mixed	whole	1	pos.	nearly equal
Orthoptera					
47. Gryllus	adult	whole	1	pos.	nearly equal
48. Ceuthophilus	"	"	1	pos.	less
49. Locusta	"	"	1	pos.	nearly equal
50. Melanoplus bivittatus	adult	"	2	pos.	nearly equal
51. Dissosteira	"	"	1	pos.	less

Total—34 species, 108 incinerations

A glance at the results in Table II shows positive reactions for copper in all insects incinerated, no matter what the stages chosen for ashing.

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This universal presence of copper among the Insecta, not only in aquatic forms, but also in terrestrial species, indicates that it has an important function which hitherto has been overlooked. Its universal distribution is certainly not adventitious. Such a contingency might be explained for aquatic insects on the basis of the food supply (a large percentage of Crustacea), but would hardly apply to terrestrial insects, especially those among the latter which feed on plants only, or whose food is even more restricted, as in the case of honey bees. Furthermore, the copper present in the blood of many insects exists in practically the same proportions as in the blood of Crustacea. In Belostoma, for instance, five cc. of incinerated blood reacted to ammonia, showing a slightly fainter shade of blue than an equivalent amount of incinerated Cambarus blood. Indeed, copper was present to such extent, that an incineration of one cc. showed decisive reactions with all the reagents listed except ammonia. Other examples might be adduced, such as wasps and ant-lions. Here the ash of a single individual gave positive response to tests for copper.

Based on the foregoing results, the writer offers the hypothesis that the rôle of copper in insects is to form the nucleus of a blood protein,—namely a hemocyanin, similar in constitution to the known hemocyanins of Crustacea and mollusks; and that it serves a similar purpose, that of a respiratory pigment.

Based on experiments, recorded elsewhere, on the presence of oxygen and carbon dioxide in the blood of insects, the writer advances the further suggestion, that the function of this hemocyanin is parallel to that of hemoglobin,—namely, that the hemocyanin carries both oxygen and carbon dioxide, that compounds are formed in the respiratory cycle similar to the oxy- and carbohemoglobins. This second hypothesis has not been proved directly, but it is logical to assume that analogy of function in a respiratory protein, as hemocyanin is analogous to hemoglobin, should result in analogous compounds during the respiratory cycle. In short, it is reasonable to assume the formation of oxyhemocyanin with oxygen, and of carbohemocyanin with carbon dioxide.

From this standpoint, the various respiratory structures of advanced aquatic insects, such as the gill filaments and gill pouches of Trichoptera larvae and aquatic caterpillars, and the others referred to in the opening paragraph of this paper, acquire a real significance. If considered as of the category of true gills, to which type they undoubtedly belong, it is easy to understand how e ective they would be with a respiratory protein. Without such a pigment to fix the gases they would seem purposeless as structures, and inefficient physiologically.

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III. Copper in Other Animals

As already stated, hemocyanin has been reported for mollusks, several species of higher Crustacea, scorpions and Limulus among Arachnida, and more recently for Coelenterates and fish. To ascertain whether or not it is found in the other classes of Arthropoda, the writer incinerated several species of plankton Crustacea, spiders, daddy-long-legs, centipeds, and millipeds (see Table III). In all of these copper was discovered in quantities equal to or exceeding the amount present in Cambarus blood.

As seen from this same table, examples of other phyla were also incinerated, including snails and slugs among mollusks, Lumbricus among Annulata, Ascaris among Nemathelminthes, Volvox among Protozoa, and human blood and snake blood for Chordata.

This material also was collected locally, except nos. 13-16 inclusive, which were collected from Wisconsin lakes, and kindly sent me by Prof. Chancey Juday, of the Wisconsin Geological and Natural History Survey.

The determination of copper in the blood of Cambarus has been noted repeatedly in this paper. The blood of this Crustacean was used constantly as a control for other incinerations. Only a small number of these controls are listed in the table. A second pigment has been reported for marine Decapoda, called Tetronerythrin, which is found also in our fresh water crayfish. The function of this pigment is unknown, altho it has been stated definitely that it is not a respiratory pigment. It is probable, however, that the pigment is used in the coloration and markings of the exoskeleton, and that it is carried passively in the bloodstream, similar to the pigments found in insect blood, and elaborated during the ecdysis. It is readily perceived in crayfish blood, from which it may be crystallized in orange-red crystals. Ordinarily it is not very abundant, but previous to moulting it is present in quantities sufficient to give a distinct pink or reddish color to the blood. Indeed, in larger crayfish, exceeding four inches in length, I have found the blood a bright red or scarlet, so that it resembled the diluted and aerated blood of a vertebrate. This blood clots in dark red masses, also resembling the clots of vertebrate blood. In "soft," freshly moulted crabs the blood appears transparent and contains little or none of this pigment.

Among species other than Arthropod Volvox furnished perhaps the greatest surprise by its show of copper, not merely as a trace, but in appreciable quantity. About 15 cc. of filtered Volvox were used in this incineration. That the reaction could not have been due to residual water is indicated by the fact that 100 cc. of water from the same pond showed not the slightest trace of this element. Its function in Volvox is problematical.

Ascaris furnished an additional surprise. Surely no one would suspect copper in an internal parasite. However, as barely a trace was found,

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TABLE III. COPPER IN ANIMALS OTHER THAN INSECTS

Name	Stage	Tissue	No. of incinerations	Result	Remarks
Crustacea					
1. Cambarus sp.	1 in. long	blood	3	pos.	
2. "	1 in. long	whole	4	pos.	
3. "	2 in. long	blood	4	pos.	More Cu. than in No. 1.
4. "	2 in. long	whole	3	pos.	" " " " No. 3
5. "	3 in. long	blood	6	pos.	" " " " "
6. "	3 in. long	whole	3	pos.	" " " " No. 5
7. "	4 in. long	blood	5	pos.	" ", " " "
8. "	4 in. long	whole	3	pos.	" " " " "
9. "	5 in. long	blood	3	pos.	" " " " No. 7
10. "	5 in. long	whole	2	pos.	" " " " "
11. Hyalella	adults	whole	2	pos.	less than No. 2
12. Cladocera & Copepods	adult	whole	1	pos.	" " "
13. Daphnia pulex	adults	whole	1	pos.	" " "
14. Microcystis	adults	whole	1	pos.	" " "
15. Copepods chiefly Limnocalanus)	adults	whole	1	pos.	" " "
16. Daphina pulex	adults	whole	1	pos.	" " "
Arachnida					
17. Argiope sp.	adult	whole	1	pos.	Equal to No. 9
18. Phalaena sp.	adults	whole	1	pos.	More than No. 9
19. Several spiders	adults	whole	1	pos.	Equal to No. 9
Myriapoda					
20. Millipeds spp.	adults	whole	1	pos.	More than No. 9
21. Centipeds spp.	adults	whole	1	pos.	More than No. 9
Annulata					
22. Lumbricus		whole	1	pos.	trace
Mollusca					
23. Physa sp.	mixed	whole	5	pos.	Equal to No. 3
24. Slugs	adult	whole	2	pos.	Equal to No. 4
Nemathelminthes					
25. Ascaris	♂ & ♀	whole	1	pos.	trace
Protozoa					
26. Volvox	mixed	whole	1	pos.	Equal to No. 2
Chordata					
27. Thamnophis sirtalis	adult	blood	1	pos.	trace in 2.8 gr.
28. Homo sapiens	adult	blood	1	neg.	about 1.6 gr. used.

and this due probably to mechanical storage, it would hardly be justifiable to attribute any physiological rôle to copper in this parasite. Its source is most probably the plant food taken in by the host.

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In snails and slugs the copper undoubtedly occupies the same rôle that it has in squids, clams, and other mollusks. Lumbricus, like Ascaris, showed only a trace. Aside from leeches, this is the first time copper has been noted for an Annelid.

The fact that an abundance of copper was found in Myriapoda and in several representatives of the Arthropoda, in some even more than in the control substance, lends definite support to the assumption that for all Arthropoda copper is an essential element, and functions in the rôle of a respiratory protein in all members of this largest of phyla.

The discovery of copper in snake blood was due to pure chance. Two and eight tenths grams of snake blood besides a small quantity of human blood had been incinerated for another purpose. While waiting to utilize the ash at some later date it occurred to the writer to test for copper. (At the time I did not know of Rose and Bodansky's discovery of copper in marine fish.) The various tests were negative except two in which the test drop had been placed under alcohol vapor for several hours. No reaction showed in the first fifteen minutes, but after that indications of a positive reaction were noticeable. Later, when examined after an interval of several hours, the reactions showed definitely positive.

The ashed human blood referred to in the foregoing paragraph was also tested, but gave negative results. However, since the quantity was even less (1.83 gr.) than the snake blood used, the experiment is inconclusive.

IV. The Sources of Copper

For aquatic animals the source of copper is the slight amount in solution in the water. It is thus that mollusks and Crustacea obtain the copper necessary to their respiration. The soluble copper originates from the soil. Since the distribution of mollusks and Crustacea is universal, copper must likewise be available universally.

For terrestrial animals such as bees, wasps, caterpillars, moths, spiders, centipeds, etc., the soil cannot be considered as a direct source of copper. Their food consists largely of plants and animals, and perhaps minute droplets of water from wayside pools. It is evident that their copper must eventually come to them by way of their plant food. To determine this positively, a number of plants were incinerated and tested for copper (Table IV).

All plants reacted positively, but only to the more sensitive reagents, as the copper is present only in traces, not at all in amounts comparable to that of Arthropoda. All parts of the plant showed the presence of copper, with this difference: the fruit generally contained a less amount than the stem, leaves, or root. Because of the minimal amounts, its rôle in the plant is

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probably not an active one, and its presence due to mechanical storage. As far as the relation of copper and plants is concerned, the copper ion is known to be highly toxic to plants, especially to the lower forms of plant life.

TABLE IV. COPPER IN PLANTS

Name	Part	No. of Incinerations	Result for Copper
1. Watermelon	Rind	2	Pos. trace
2. Pear	Leaves	2	pos. trace
3. Pear	fruit	2	pos. less than No. 2
4. Tomato	leaves and stem	2	pos. trace
5. Tomato	fruit	2	pos. less than No. 5
6. Potato	leaves	1	pos.
7. Lettuce	leaves	2	pos.
8. Red Beet	leaves	2	pos.
9. White Beet	leaves	2	pos.
10. Apple	leaves	2	pos.
11. Apple	fruit	2	pos. less than No. 11
12. Currant	leaves	2	pos.
13. Celery	stem	2	pos.
14. Clover	heads	1	pos.
15. Clover	leaves	2	pos. more than No. 14

Total—11 species, 30 incinerations.

Here in Moscow the copper content of the soil is very low. The surface soil is of aeolian origin and what copper it contains is brought by dust-storms from mountains 100 to 200 miles to the west in Washington and Oregon. In a quantitative estimate of copper in the soil 50 grams yielded only sufficient copper to permit a qualitative test.

Samples of water, each 3500 cc., taken from Paradise Creek during the summer and concentrated to 5 cc., likewise showed little more than traces of copper. A sample of water taken more recently (November) from under the ice showed a copper content of 0. 0187 gr. by the sulphocyanide method or approximately 0.00534 gr. per liter. The mud of Paradise Creek shows somewhat larger amounts, some of which may be due to organic matter.

V. DISCUSSION

In a recent paper on the occurrence of copper in marine organisms, Rose and Bodansky (1920) note the previous demonstration of copper in the following groups:

1. Echinodermata—starfish, urchins, sea-squirts.
2. Annulata—leech.
3. Crustacea—various Decapoda.
4. Arachnida—Scorpion, Limulus.

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5. Mollusca—clams, oysters, snails, cuttle-fish, octopus.
6. Tunicata—Ciona.
7. Pisces—shark, 2 Teleosts.

To this list Rose and Bodansky add:

1. Coelenterata—jellyfish, Portuguese Man-of-War.
2. Mollusca—oysters, clams.
3. Curstacea—shrimps and crabs.
4. Pisces—Torpedo and sting ray, 12 Teleosts.

In all they add some 35 species, demonstrating for the first time that the copper in fish is not due to pathological causes. A survey of the groups studied in the present paper shows some interesting additions to the foregoing lists:

1. Protozoa—Volvox.
2. Nemathelminthes—Ascaris.
3. Mollusca—snails, slugs.
4. Annulata—Lumbricus.
5. Arthropoda.
   a. Crustacea—plankton, Cambarus, Hyalella.
   b. Arachnida—Phalaena, spiders.
   c. Myriapoda—centipeds, millipeds.
   d. Insecta—13 chief orders, over 35 species.
6. Chordata—snake.

To these must be added the occurrence of copper in higher plants.

Such a wide distribution of an element in a variety of living organisms, representing eight of twelve phyla, must have some significance. Its occurrence cannot be wholly adventitious, especially since it may be present in considerable quantities in the organism. Where present only in traces, it may well be ignored. In a number of forms its physiological rôle has been known for some time, altho physiologists believed that it was restricted to a few scattered species, and really was more or less an abnormality or rarity. Schulz, for instance (in Abderhalden 1910), states, "Hemocyanin occurs in the blood of higher Crustacea. It is present *only in a few members* (italics mine) of this class (Homarus, Maja, Portucuco, etc.)" Yet it is evident from the work of Rose and Bodansky and from the experiments herein noted that copper is not at all restricted to a few Decapoda among Crustacea, but that even the simplest and smallest Crustacea contain it.

Indeed, the writer, once he found positive indications of copper in a few species of insects, set out to test representatives of as many different groups as he could obtain locally. These were taken wholly at random, representing a variety of living conditions, from aquatic to parasitic, and entirely without regard to possible favorable results. (As a matter of fact,

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in the end I purposely selected some of the least likely animals, such as Volvox, Ascaris, Lumbricus, and snakes.) This same attitude held for the work on lower Crustacea, spiders, and so on. The results, I believe, more than justified such a procedure, by indicating copper in the most unexpected places.

Yet this very fact of random selection is all the more convincing in a general application of the phenomena discovered. It signifies that these random selections are representative of whole groups and that what pertains to the few pertains also to the many. Since in these representative species copper has been found, and since in at least some of these few the physiological rôle of the copper has been definitely ascertained, it can be concluded that all or most remaining members of these groups also possess copper and that its physiological rôle is also similar. In other words, in at least the Mollusca and Arthropoda all species contain copper in appreciable quantities, and this copper functions as the nucleus of a respiratory protein, hemocyanin.

In thus extending particulate findings to entire groups of organisms I do not think I am overstepping the bounds of proper scientific conservatism. For copper was found in many groups, while in certain groups where a greater variety of material was available every species studied showed positive results. The uniformity of the results in these groups, unexpected as they were, is convincing and, I believe, warrants the above generalization.

VI. Summary

1. Both oxygen and carbon dioxide are present in insect blood in appreciable quantities. Insect blood aids in the transportation of gases, and the respiratory function is not confined to the tracheae.

2. Over 100 incinerations were made of insect blood, or whole specimens, and the ash tested for copper. Copper was found in all cases. The 35 species studied represent 13 of the chief orders of insects, in both larval and adult stages.

3. Copper is found in insect blood in quantities comparable to that of crayfish blood. Its rôle is therefore interpreted as being identical,—namely that it serves as the nucleus of a respiratory protein,—hemocyanin. Insects, therefore, have two sources of oxygen,—atmospheric air led directly to the tissues by way of the tracheae, and fixed oxygen carried by the respiratory protein of the blood.

4. Incinerations of plankton Crustacea, spiders, and centipeds gave positive results for copper, showing that copper is distributed among all classes of Arthropoda. It may therefore be regarded as an element essential to the physiological activity of Arthropods, its rôle being to act in a respiratory pigment for all members of this phylum.

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5. As representatives of other phyla Volvox, Ascaris, snails and slugs, Lumbricus, human and snake blood were incinerated. All of these, except human blood, showed varying amounts of copper.

6. As sources of copper the water, soil, and plants were tested. All plant ash showed traces of copper. The water samples of this region showed only small quantities, while the soil showed varying amounts.

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

MICROSCOPE ILLUMINATION WITH REFERENCE TO BROWNIAN MOVEMENT AND COMBINATION LIGHTING[1]

By

Alexander Silverman

Brownian Movement.—For the study of this phenomenon china clay was mixed with distilled water and passed through a double quantitative filter paper. The opalescent filtrate was run into the cavity of a hollow-ground slide, and this in turn placed over dull black paper on the stage of the microscope. A 4mm. apochromat objective surrounded by a ring-lamp[2] was lowered into the solution and the instrument focused.

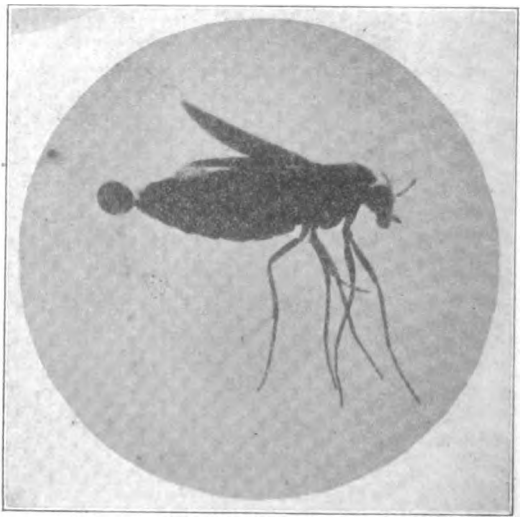

Fig. 1

[1] Published by courtesy of the American Chemical Society. Read before the Division of Industrial and Engineering Chemistry at the Rochester, N. Y. meeting of the American Chemical Society, April 25–29, 1921.

[2] J. Ind. Eng. Chem., 9(1917), 971; 10(1918), 1013; 12(1920), 1200.—J. Soc. Chem. Ind., 38(1919), 126.—J. Royal Mic. Soc., No. 253 (1920), 98.

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A 10x compensating ocular gave a magnification of 440 diameters. Not only was Brownian movement clearly evident, but the dot-like, rod and lenticular shapes of particles were shown with great definition in white against a black background.

COMBINATION LIGHTING.—A comparative study was made of (1) the effect of transmitted light, (2) of direct super-stage illumination from a ring-lamp surrounding the objective, and (3) of combination lighting from above by the same lamp, a part of whose light passed through a glass slide and was reflected back by a sub-stage mirror placed parallel to the stage.

For this study a fossil insect in amber was employed. The length of the insect, 0.9 mm., will enable the reader to judge the magnification in the illustrations. A combination of 32 mm. objective and 10x ocular together with the length of bellows used in photographing gave a magnification of 60 diameters in the original photographic prints.

Figure 1 shows the effect of transmitted light from below, placing the mirror at an angle and using a 25 watt frosted spherical lamp. While sufficient contrast is obtained details are lacking because of the varying thickness of the insect.

Figure 2 shows the effect of direct light from the ring-lamp above the object with dull black paper placed under the slide. Some details are visible, but there is no contrast Beautiful detail may be obtained by substituting white paper for black.

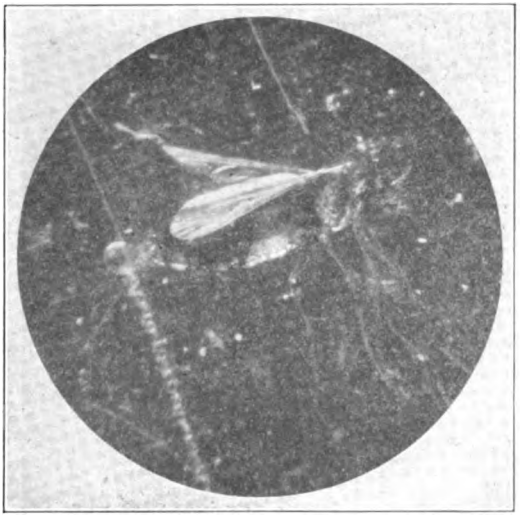

FIG. 2

Figure 3 is the result of a combination of light from the ring-lamp above, with its own light reflected upwards by the sub-stage mirror placed parallel to the microscope stage. A black paper was held over the sub-stage mirror after 15 seconds. This

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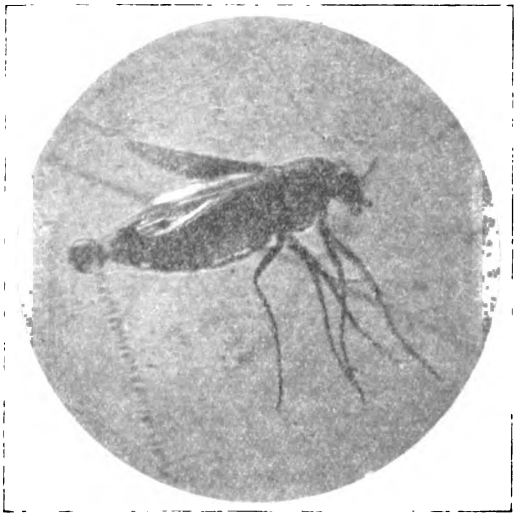

FIG. 3

Department of Chemistry,
University of Pittsburgh.

affords contrast and detail, and the method may prove desirable for photographing an object which has transparent, translucent and opaque parts.

Seeds Graflex plates, exposed 60 seconds, with a Davies shutter closed to the smallest stop, gave the results obtained in the insect photographs.

Conclusions.—This paper emphasizes the importance of a study of background colors and of combination lighting in microscopy and cites specific examples.

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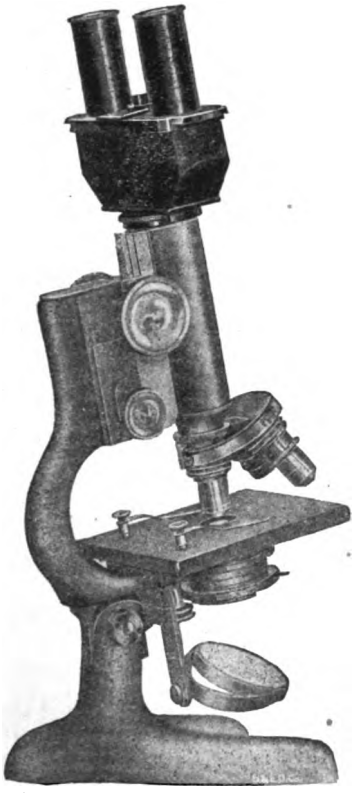

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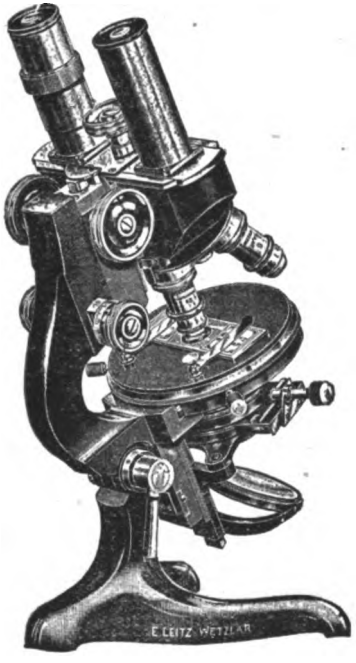

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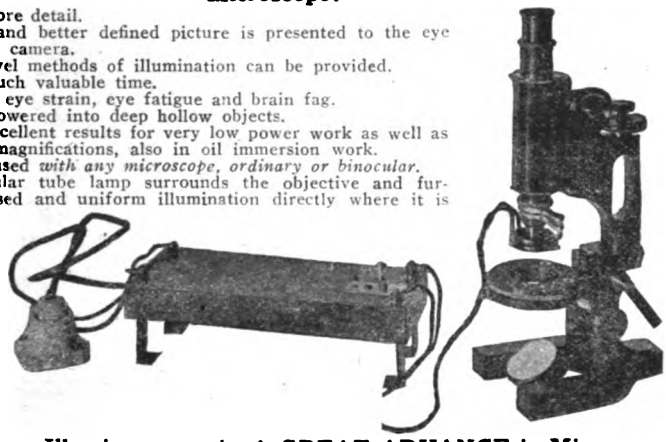

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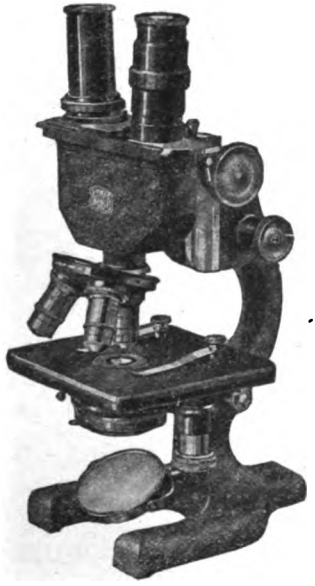

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INDEX TO ADVERTISEMENTS

Anglers Co., Chicago, Ill..... III
Bausch and Lomb Optical Co., Rochester, N. Y..... V
Comstock Publishing Co., Ithaca, N. Y..... III
General Biological Supply House, Chicago, Ill..... II
George Banta Publishing Co., Menasha, Wis..... III
Guide to Nature, South Beach, Conn..... IV
Hubert, H. Edw., New Orleans, La..... II
Kny-Scheerer Corporation of America, New York..... IV
Leitz, E., New York..... VI
Ludwig Hommel & Co., Pittsburg, Pa..... VII
Marine Biological Laboratory, Woods Hole, Mass..... I
Powers and Powers, Lincoln, Nebr..... I
Southern Biological Supply Co., New Orleans, La..... I
Spencer Lens Co., Buffalo, N. Y..... VII
Sphung, A. A., North Judson, Ind..... I
Ward's Natural Science Establ., Rochester, N. Y..... III
Watson & Sons, London, Eng..... VIII

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1921

VOLUME XL NO. 4

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TABLE OF CONTENTS

For Volume XL, Number 4, October, 1921

On the Effect of Some Fixatives upon Myxosporidian Spores, with four figures, by R. Kudo 161

New Species and Collections of Arrhenuri: 1921, with three plates by Ruth Marshall 168

Some Work on Marine Phytoplankton in 1919, by W. E. Allen 177

The Accessory Chromosome of Anasa tristis again, by A. M. Chickering 182

DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

The Literature of Diatoms, by Fred B. Taylor 187

A Compendium of the Hosts of Animal Parasites contained in Ward and Whipple's Fresh-water Biology, compiled by H. J. Van Cleave 195

The Endocrines, by S. W. Bandler, reviewed by T. W. Galloway 200

List of members 206

Index 207

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)
Vol. XL OCTOBER, 1921 No. 4

ON THE EFFECT OF SOME FIXATIVES UPON MYXOSPORIDIAN SPORES[1]

By
R. Kudo

Although it has generally been recognized that when myxosporidian spores are fixed, stained and mounted as either smears or as section preparations, they appear smaller than in the fresh state, it was Cépède who first called attention to the matter. He recognized differences between fresh and stained spores of the species he studied, and concluded as follows (Cépède, 1906:63): "en présence de telles différences de taille et de l'importance donnée actuellement aux dimensions des spores des Myxosporidies dans la systématique comme caractère distinctif des espèces, je crois utile de faire remarquer qu'il serait bon d'indiquer si les mensurations des spores ont été faites in vivo ou sur des préparations fixées et colorées, et montées au baume."

My observations upon the species which I have studied up to date agree with Cépède's results and have suggested that the dimensions of spores of a species should be accompanied by the statement of conditions under which the measurements were made (Kudo, 1920:49).

Most authors agree that at the present state of our knowledge regarding this group of Protozoa, a satisfactory classification of genera and species of Myxosporidia, must have as its basis the study of the spore (Kudo, 1920:52-59). The size, dimensions and structure of spores show a certain amount of variation even in one species, yet they are far more typical of the species than are the vegetative forms. In every case, the identification of a species has been successfully done only when the spores were present.

Since the characters of the spore vary to a more or less recognizable extent, according to the difference of conditions under which the spores are observed, it naturally follows that the characters of spores of two different species can only be correctly compared when they are observed

[1] Contributions from the Zoological Laboratory of the University of Illinois, No. 191.

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under exactly the same conditions. In other words, if the spores were originally observed and measured in the fresh state, those of the species to be compared with the former, should also be studied in the same condition. This kind of comparison, however, has happened only in a few cases, and can not always be carried out even in future. Many species of Myxosporidia have accidentally been found and described from stained smears or section preparations only by several authors, who were engaged with studies on other topics. Furthermore, even if one deals exclusively with Myxosporidia, one is frequently compelled to omit the study of fresh spores and to confine oneself to that of stained preparations under various circumstances.

The characters of spores observed only in section preparations can, of course, not only be compared properly with those obtained from fresh spores, but also should not be used for the data of establishing a new species. Special precaution must be exercised in cases where two forms are similar in habitat and locality and whose vegetative stages are not known.

Recently Schuurmans Stekhoven (1920) described three new species of Myxosporidia, *Sphaerospora gasterostei*, *Myxidium rhomboideum* and *Henneguya renicola*, found in the uriniferous tubules of the kidney of *Gasterosteus pungitius* (misprinted as *pungiticus*) from Holland. The author studied section preparations of the host kidneys which were fixed with 60 per cent. alcohol and stained with Delafield's hematoxylin, and compared the characters of the spores observed therein with those of already known three species, *Sphaerospora elegans*, *Henneguya media* and *Henneguya brevis* (Kudo, 1920:30). Thélohan (1895) who described these latter forms, seems to have studied them in both fresh and fixed conditions, although his description is unfortunately brief. When the forms are so similar in every respect except the size of the spores, one finds it extremely difficult to decide whether the newly observed species studied only in sections, are identical with the former or not. If, however, we can calculate the dimensions of spores in the fresh state from those obtained from the stained ones, we can undertake a more satisfactory comparison between the species observed under diverse conditions.

In order to see exactly how the fixation, staining and mounting would affect the shape, dimensions and structure of Myxosporidia, a few experiments were performed on the spores of *Leptotheca ohlmacheri*. This Myxosporidian was first found by Ohlmacher and Whinery (1893) in the kidney of *Bufo lentiginosus*. I have recently observed it in the kidneys of *Rana clamitans* and *Rana pipiens*. Although I formerly placed it provisionally in the genus Wardia (Kudo, 1920:83-84), my recent study on its morphology and life history which will be published elsewhere, has shown that it should be placed in the genus Leptotheca as Labbé suggested.

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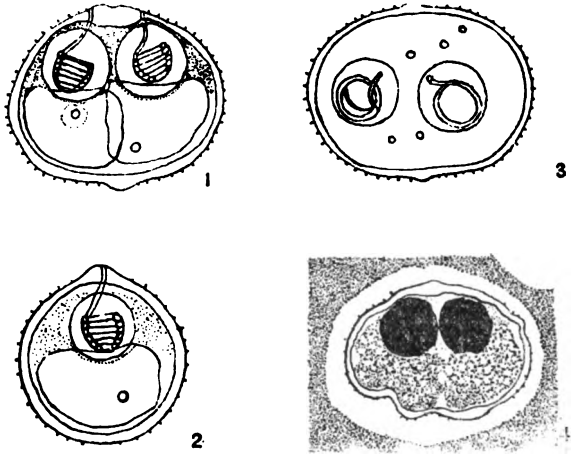
The fresh spore of *Leptotheca ohlmacheri* (Figs. 1 to 3) is oblong with its longest diameter at right angles to the sutural plane. The anterior end is slightly attenuated due to the thickening of the spore membrane at that point, while the posterior end is rounded. In profile, it is nearly circular with the slightly attenuated anterior extremity. In the anterior end view, it is regularly oblong. The shell is moderately thick. The sutural



Spores of *Leptotheca ohlmarcheri* (Gurley) Labbé. Figs. 1, 2 and 3. Three optical sections in front, side and anterior end views respectively of fresh spores which were kept in a hanging drop preparation with physiological solution and which are typical of the species in form, structure and dimensions. Fig. 4. A spore from a smear fixed with absolute alcohol, stained with Giemsa stain and mounted in cedar oil, showing the shrinkage of the entire body. All about X 2100.

ridge is well marked and protrudes conspicuously at the ends. The spore membrane is somewhat irregularly striated. Three to seven striæ run parallel to the sutural line on each valve, the remaining ones make somewhat similar angles with the former. The striæ in lateral view are mostly placed horizontally. The number of striæ on each valve varies from 25 to 35. Two spherical polar capsules usually equal in size in one spore, occupy the anterior portion of the spore. The polar filament, coiled 4 to 6 times, is distinctly visible. Two independent sporoplasms occupy the extracapsular cavity of the spore. They are extremely homogeneous and each to be the karyosome of the nucleus). The size of the spore varies to some extent. There are some larger and some smaller than the average spores as is the case with every species found, doubtlessly due to the malformation. The average dimensions are as follows:

Sutural diameter	9.5 to 12μ; average	10.8μ
Breadth	13 to 14.5μ; average	13.75μ
Thickness	9.5 to 12μ; average	10.8μ
Diameter of polar capsule	3.5 to 4.5 μ; average	4.0μ
Length of polar filament (KOH)	42 to 62μ; average	52.0μ

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A drop of the emulsion of fresh spores in physiological salt solution was smeared on a slide. The amount of the emulsion and the area over which it was smeared were made approximately the same in every smear so as to obtain the similarity in the number of spores and the conditions under which the spores existed until they were fixed. Soon after the smear was made, it was fixed in one of the following fixatives before it dried up one smear being purposely made to dry up: 50 per cent., 70 per cent. and absolute alcohols, 4 per cent. formol, Schaudinn's (warmed), Bouin's and Flemming's (weak) mixtures. The fixatives were allowed to act upon the smears for 16 hours, after which they were removed from the latter by proper washings. At the same time, small pieces of the infected host organ were fixed in Schaudinn's fluid and 4 per cent. formol respectively, sectioned in paraffin and stained with Heidenhain's iron hematoxylin. The fixed smears were stained with Heidenhain's iron hematoxylin, Delafield's hematoxylin or Giemsa stain, and mounted in Canada balsam or cedar oil (for Giemsa stained smears only).

From each of the preparations, one hundred mature spores which could be distinguished distinctly from those that are in the course of development, were drawn at a scale of 2,100 magnification, and measured. The results are as follows:

		Sutural Diameter			Breadth			Diameter of Polar Capsules		
		Range in μ	Average in μ	Loss in μ	Range in μ	Average in μ	Loss in μ	Range in μ	Average in μ	Loss in μ
SMEARS	Fresh spores (control)	9.5–12.0	10.8	0	13–14.5	13.75	0	3.5–4.5	4.0	0
	Air-dried, unstained, alcohols and balsam	7.6– 9.5	8.6	2.2	10.5–11.4	10.7	3.05	2.6–3.2	2.9	1.1
	50% alcohol; Giemsa	9.2–10.0	9.6	1.2	10.5–10.9	11.0	2.75	3.0–4.0	3.5	0.5
	70% alcohol; Delafield	9.0– 9.8	9.4	1.4	9.8–10.9	10.4	3.35	2.8–3.9	3.4	0.6
	Absolute alcohol; Giemsa	9.0– 9.5	9.25	1.55	9.5–10.9	10.2	3.55	2.8–4.0	3.4	0.6
	4% formol; Giemsa	8.9– 9.3	9.1	1.7	9.5–11.4	10.5	3.25	2.9–3.8	3.4	0.6
	Schaudinn; Giemsa or Heidenhain	9.2– 9.8	9.6	1.2	10.9–11.9	11.4	2.35	3.0–3.8	3.4	0.6
	Bouin; Heidenhain	8.5– 9.5	9.0	1.8	9.5–10.9	10.2	3.55	2.6–4.0	3.3	0.7
	Flemming; Heidenhain	8.8– 9.6	9.2	1.6	10.0–11.2	10.6	3.15	2.4–3.2	3.3	0.7
	Average	.....	9.2	1.5	.....	10.6	3.09	.....	3.4	0.6
	Per cent. of loss, calculated from the dimensions of spores from smears	.....	.....	16.0%	.....	.....	29.0%	.....	.....	17.0%
SECTIONS	Schaudinn; Heidenhain	8.6–10.0	9.3	1.5	10.6–11.8	11.1	2.65	3.0–3.6	3.3	0.7
	4% formol; Heidenhain	8.4– 9.9	9.15	1.65	9.5–11.3	10.4	3.35	2.8–3.8	3.3	0.7
	Average	.....	9.23	1.58	.....	10.75	3.00	.....	3.3	0.7
	Per cent. of loss, calculated from the dimensions of spores in sections	.....	.....	16.0%	.....	.....	28.0%	.....	.....	21.0%

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From the above table, the following may be noted:

1) The amount of loss in sutural diameter of the spore of *Leptotheca ohlmacheri* is greatest when the spore is airdried and smallest when it is fixed with either Schaudinn's fluid or 50 per cent. alcohol. The average loss amounts to about 14 per cent. of the sutural diameter of the fresh spores.

2) The amount of loss in breadth of the spore is greatest when the spore is fixed with Bouin's fluid or absolute alcohol and smallest when it is fixed with Schaudinn's fluid. The average loss amounts to about 22 per cent. of the breadth of the fresh spores.

3) The amount of loss in the diameter of polar capsule is greatest when the spore is air-dried and smallest when it is fixed with 50 per cent. alcohol. The average loss amounts to about 15 per cent. of the diameter of polar capsules in fresh spores.

4) The losses in smear and section preparations are almost similar.

In the case of *Myxobolus cycloides*, Cépède gave the following dimensions: Fresh spores: sutural diameter, 13.5-16μ, breadth 11-13μ. Schaudinn-Heidenhain's spores: sutural diameter 10.5-12μ, breadth 7.5-8μ. Thus in this case, the fixation caused loss of 3.5μ and 4.25μ respectively in sutural diameter and breadth of the spore. These losses amount to 25 and 35 per cent. compared with the fresh spores.

The loss in the latter species is greater than the former species. It, however, shows distinctly from these two different types of Myxosporidia, that the sutural diameter undergoes a smaller amount of shrinkage than the breadth.

Concerning the change in the dimensions of spores by fixation, Cépède states that the reason why the spores appear smaller in fixed and stained conditions than in fresh condition, is simply because the refractive power of Canada balsam makes the unstained spore membrane invisible, and not because the shrinkage of the body caused by fixation takes place.

In the case of *Leptotheca ohlmacheri*, this is not the case. Very frequently spores such as shown in Fig. 4, are seen in the smears. The spore undoubtedly occupied the entire area which appear as blank zone before it was fixed. When fixed, the contents underwent a strong shrinkage, thus leaving a clear unstained zone between it and the smear. In such a spore, one can distinctly see the spore membrane in an irregular outline. I have noticed similar change of spores in smears of many species from various genera suggesting shrinkage as the main cause for the loss in dimensions. I, therefore, consider the decrease in the dimensions of myxosporidian spores, in general, is caused by the shrinkage of the entire spore body under the influence of the fixative and subsequent treatments.

The amount of shrinkage caused by fixation will apparently be different in different genera and species. Unless a large number of measurements

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on different species be made, we do not know exactly the data on which the dimensions of fresh spores of the species observed by Schuurmans Stekhoven may correctly be calculated. Assuming that the spores of *Sphaerospora gasterostei* and *Henneguya renicola* underwent shrinkage similar to that of *Leptotheca ohlmacheri*, we obtain the following comparison:

	Sphaerospora gasterostei		*Sphaerospora elegans*
	Schuurmans Stekhoven	Calculated	Thélohan
Length	6.7 μ	7.8 μ	10 μ
Breadth	7.0 μ	9.0 μ	11 μ
Length of polar caps.	3.5 μ	4.1 μ	——

If the calculation is correct, it seems probable that *Sphaerospora gasterostei* is independent from *S. elegans*.

	Henneguya renicola		*Henneguya media*	*Henneguya brevis*
	Schuurmans Stekhoven	Calculated	Thélohan	Thélohan
Length of spore	8 μ	9.28 μ	20–24 μ (?)	10 μ
Breadth	3.5 μ	4.5 μ	5–6 μ	5–6 μ
Polar capsule (length)	4.5 μ	5.4 μ	4–5 μ	4–5 μ
Tail	15 μ	17.4 μ	——	14–15 μ

The calculated value of *Henneguya renicola* resembles closely the dimensions given by Thélohan for *Henneguya brevis*. The form of spore becomes so highly modified in section preparations that it is hard to make out the form in the fresh state. Therefore, it is highly doubtful whether Schuurmans Stekhoven saw a new species or not.

The form of spores changes to a variable extent according to the difference of the fixatives used. The more shrinkage the spore undergoes, the more irregular outlines it assumes. Careful fixation in Schaudinn's fluid often preserves the form of spores very nicely.

When a spore is fixed with any one of the fixatives, the coiled polar filament becomes entirely invisible. This is probably caused by the coagulation of the wall of polar capsule which becomes opaque by the

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fixation. The distinction between the two sporoplasms is harder to determine in fixed and stained spores than in fresh spores. The sporoplasms become coarsely reticulated, losing the homogeneous condition seen in the fresh state.

In conclusion, I may again suggest that a new species should be described after studying the spores in fresh as well as fixed and stained conditions and if possible the fixed vegetative forms.

Summary

1) The ordinary fixation causes about 14 and 22 per cent. decrease respectively of the sutural diameter and breadth of fresh mature spores of *Teptotheca ohlmacheri*.

2) The possibility of calculating the dimensions of fresh spores from those of fixed and stained spores is discussed.

3) The decrease in the dimensions of spores is due to the shrinkage of the entire spore body.

4) Fixation makes the coiled polar filament invisible.

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NEW SPECIES AND COLLECTIONS OF ARRHENURI: 1921.

By
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The genus Arrhenurus, the largest group of the water mites, continues to yield new material from collections in lake regions. The new species described in this paper came from regions as far apart as Canada and China; while additional notes on already described species are based on material secured in several states of northeastern United States, some of them from new localities. Through the kindness of Professor N. Gist Gee, of Soochow University, material was secured for the description of new species from China. Professor Frank Smith and Dr. H. R. VanCleave, of the University of Illinois, were good enough to contribute some material from Michigan, New York and Massachusetts. Through the interest of Dr. R. A. Muttkowski an opportunity was given for the examination of some collections of the Biological Station of the United States Bureau of Fisheries at Fairport, Iowa; and more recently the author was privileged to see some collections of Dr. F. A. Stromsten, of the University of Iowa. The author's own collections from the Muskoka Lake region of Ontario, together with other material from various sources, form the basis of a preliminary account of the genus Arrhenurus as it has been found in Canada. This topic will be discussed first.

Practically the only account of the water mites of Canada so far is that contained in a paper by Dr. F. Koenike, "Nordamerikanische Hydrachniden," and a revision of this paper, "A Revision of my 'Nordamerikanische Hydrachniden.'" The descriptions were based upon material sent to him by Dr. J. B. Tyrell, of Toronto, and were collected in Alberta and British Columbia, near the international line. Of the thirty species listed by Dr. Koenike, four were Arrhenuri and new species. These were *A. lautus*, *A. interpositus* (a young male), *A setiger* and *A. krameri*. The last named species has since been found by the author and further notes are given in this paper. In addition to this, one new species is now added for Canada (*A. uniformis* nov. spec.), and four more are recorded for the first time, as follows. *A. americanus* Mar. and *A. manubriator* Mar. were found by the author at Parry Sound and *A. americanus* var. *major* Mar. in a small lake near Bala, Ontario. *A. marshalli* Piers., the most widely distributed species, had previously been found in material from Long Point, Canada, in some collections of the United States Fish Commission.

In the descriptions of species which follow the Canadian material will be discussed first.

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Arrhenurus uniformis nov. spec.
Pl. IX, fig. 1-3.

This species resembles *A. scutiliformis* Mar. and belongs in the group of "long-tailed" Arrhenuri in which the very long and rather simple appendix is decidedly narrower at the end than it is at the base. The outline of the body is approximately circular; the enclosed dorsal area and the appendix are moderately high and rounded. Details of structure given in the figures show this to be a new species. The last joint of the fourth leg is long and slim; the spur on the fourth joint is moderately developed.

The single male on which this description is based is 1.33 mm. long and 0.73 mm. wide. The color is dull olive green. It was found in a small lake near Long Lake, at Bala, Ontario, August 25, 1920.

Arrhenurus krameri Koenike..
Pl. IX, fig. 7-9.

The author has already recorded (1908) the finding of a mite from Oregon which appeared to be *A. krameri*. Drawings of this specimen are now given for the first time and its identity with the single male from British Columbia on which Dr. Koeinke's description was based seems to be established. It is slightly smaller, however, measuring only 1.29 mm. A dorsal view is shown, which did not appear in the original paper, together with the lateral view and a drawing of the palpus.

Arrhenurus simulans nov. spec.
Pl. X, fig. 17-21.

Material sent to the author by Dr. H. J. Van Cleave from collections in Dump Lake, Woods Hole, Massachusetts, contained sixty individuals of this species. It was at first thought to be *A. krameri*, altho a larger form, the length being 1.45 mm. and the extreme width, 0.83 mm. The males bear some resemblance also to *A. rectangularis* Mar., especially when a comparison is made of the side views of the long appendix, the end of which in the three named species shows a double scallop, one part above the other. The body of the new species is conspicuously elevated where it joins the appendix. The wing-shaped genital areas are rather small and the ends of the line enclosing the dorsal area are far behind them on the appendix. Twenty-seven males were present. The color in the preservation is dull brown green.

Over half of the individuals in the collection were females; the examination of the palpi shows that they belong to this species. The body of *A. simulans* fem. is broadly ovate. The epimeral plates are relatively small; the third and fourth have about the same width throughout, the two posterior groups being well separated from each other and from the genital area. The fourth epimera are narrow, scarcely wider than the third. The genital plates are of nearly uniform width and extend straight out from the aperature, which is large. The body is 1.32 mm. in length.

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Arhenurus pseudosetiger nov. spec.

Pl. IX, fig. 4-6.

In a former paper (1910) the author identified as *A. setiger* Koen. an individual from Madison, Wisconsin. But a more careful study of this specimen shows that it belongs to another, though closely related species, which will be designated as *A. pseudosetiger*. The body proper is stouter than it is shown in Dr. Koenike's figures of the Canadian species, being nearly circular in outline, not oblong, and the appendix is smaller. The dorsal enclosed area runs over on the appendix and is depressed. The entire length of the body is 0.8 mm., the greatest width, in the region of the fourth leg, 0.7 mm. The color is deep brick red.

Arrhenurus trifoliatus Marshall

Pl. IX, fig. 10-12.

It is not often that the collector succeeds in securing a large number of Arrhenuri at any one time; it is still more unusual to find any one species in numbers large enough to make possible a thorough examination of all structures and to identify with certainty the females of the species. Collections made by the author in the marshy sloughs at Burlington, Wisconsin, July 5, 1919, consisted largely of individuals of the rather uncommon species, *A. trifoliatus*, twenty-five males and fifteen females being secured. It is now seen that the earlier description of the species (1908) did not show completely the details of the appendix of the male. As seen in Fig. 10, a young male, a delicate bladder-like structure, A, is attached to either side of the stout petiole, a structure which is easily in ured in preservation.

The female, which is now described for the first time, is broadly oval in form. The posterior groups of epimera are close together and the genital area lies immediately behind them. The genital wing-shaped areas are unusual in form, the outer ends curving strongly upward. The length of the body is 1.15 mm., the extreme width, 1.05 mm.

A. major was represented in this Burlington collection by one male. The Lake Beulah region which was visited at the same time yielded four species of Arrhenuri: *A. marshalli*, *A. megalurus*, *A. americanus* and *A. reflexus*, the latter having the unusual color of orange red.

Arrhenurus compactilis Marshall

Pl. X, fig. 13-16.

This somewhat rare species was found in collections from Fairport, Iowa, and again in the collections of Professor Frank Smith from Douglas Lake, Michigan. This new material adds two states to the range of the species and makes possible a more complete study of its structure. Drawings of the palpus and the last leg are now given. The latter is seen to be very characteristic of this group of stout bodied petiolated Arrhenuri in having a long fourth joint with a conspicuous spur ending in a tuft of curved hairs, while the fifth and sixth segments of this appendage are short.

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The female of the species is now known from the study of the palpi which agree in all details with those of the male except that they are somewhat larger, as usual. The body is stout and oval and measures 1.3 mm. in length. Details of the dorsal and ventral surfaces are shown in the figures.

The collections of water mites from the Biological Station at Fairport, summer of 1917, have already been referred to. They were secured from small lakes in the vicinity of the Station and were rich in Arrhenuri. This material is especially interesting since there have been no previous records of Arrhenuri from Iowa that the author is aware of. Nine species were found, as follows:

A. marshalli Piers.
A. americanus Mar.
A. americanus var. *major* Mar.
A. apetiolatus Piers.
A. birgei Mar.
A. fissicornis Mar.
A. compactilis Mar.
A. laticaudatus Mar.
A. dentipetiolatus Mar.

The last named species is rare, only two other specimens, from Colorado, having been recorded.

The collections of Dr. F. A. Stromsten, mentioned in the introduction, add *A. lyriger* Mar. to the list.

The following descriptions of two new species of Arrhenuri from China continue the study of the water mites of the region of Soochow which was begun in a former paper (1919), and are made possible through the continued interest of Professor N. Gist Gee who furnished the material.

Arrhenurus soochowensis nov. spec.
Pl. XI, fig. 22-25

The new species belongs to a type of Arrhenuri which is seen in *A. kraepelini* Koen. described from Java, a type apparently common in the Asiatic members of the genus. This is the type of *A. forpicatus* Neum. of Europe, represented in America by *A. lyriger* Mar. It is characterized by a deep incision in the end of the appendix, the hyaline appendix lying on the dorsal side of this.

The single individual upon which this description is based has these characteristics well marked. The appendix is relatively long; the median incision runs into a round opening over which lies the large and highly developed hyaline structures, closely resembling the same parts in *A. limbatus* Koen. of Madagascar. The dorsal enclosed area of the body is rather small and the line which encloses it is not quite closed. The fourth joint of the fourth leg has a short spur. The fourth joint of the palpus is unusually broad at the distal end. This is a small mite, 0.8 mm. long and 0.5 mm. wide. The color is dull green. The specific name refers to the locality where it was found.

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Arrhenurus geei nov. spec.

Pl. XI, fig. 26-29

This species resembles *A. madaraszi* Daday found in Ceylon and belongs to the *forpicatus* group mentioned under the last species. The appendix is small and narrow but well developed; its median incision is large at the upper end. The hyaline appendix is an oblong structure; on either side of it is developed a delicate claw-like piece. The body is broad; the enclosed dorsal area is large, the line enclosing it not quite closed posteriorly. The fourth leg lacks the spur on the fourth joint. The palpus, as in the related species, has a broad fourth joint. The single male on which this description is based is 0.73 mm. long and 0.56 mm. broad. The species is dedicated to Professor Gee.

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Explanation of the Plates

Plate IX

1. Arrhenurus uniformis, dorsal view.
2. Arrhenurus uniformis, genital area.
3. Arrhenurus uniformis, lateral view.
4. Arrhenurus pseudosetiger, dorsal view.
5. Arrhenurus pseudosetiger, lateral view.
6. Arrhenurus pseudosetiger, left palpus
7. Arrhenurus krameri, dorsal view.
8. Arrhenurus krameri, lateral view.
9. Arrhenurus krameri, left palpus.
10. Arrhenurus trifoliatus, appendix of young male.
11. Arrhenurus trifoliatus, female, epimera.
12. Arrhenurus trifoliatus, right palpus.

Plate X.

13. Arrhenurus compactilis, female, dorsal view.
14. Arrhenurus compactilis, female, ventral view.
15. Arrhenurus compactilis, male, part of left fourth leg, the last three joints rotated.
16. Arrhenurus compactilis, right palpus.
17. Arrhenurus simulans, female, epimera.
18. Arrhenurus simulans, male, dorsal view.
19. Arrhenurus simulans, appendix, ventral view.

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20. Arrhenurus simulans, lateral view.
21. Arrhenurus simulans, left palpus.

Plate XI.

22. Arrhenurus soochowensis, dorsal view.
23. Arrhenurus soochowensis, lateral view.
24. Arrhenurus soochowensis, ventral view.
25. Arrhenurus soochowensis, left palpus.
26. Arrhenurus geei, lateral view.
27. Arrhenurus geei, dorsal view.
28. Arrhenurus geei, appendix, ventral view.
29. Arrhenurus geei, left palpus.

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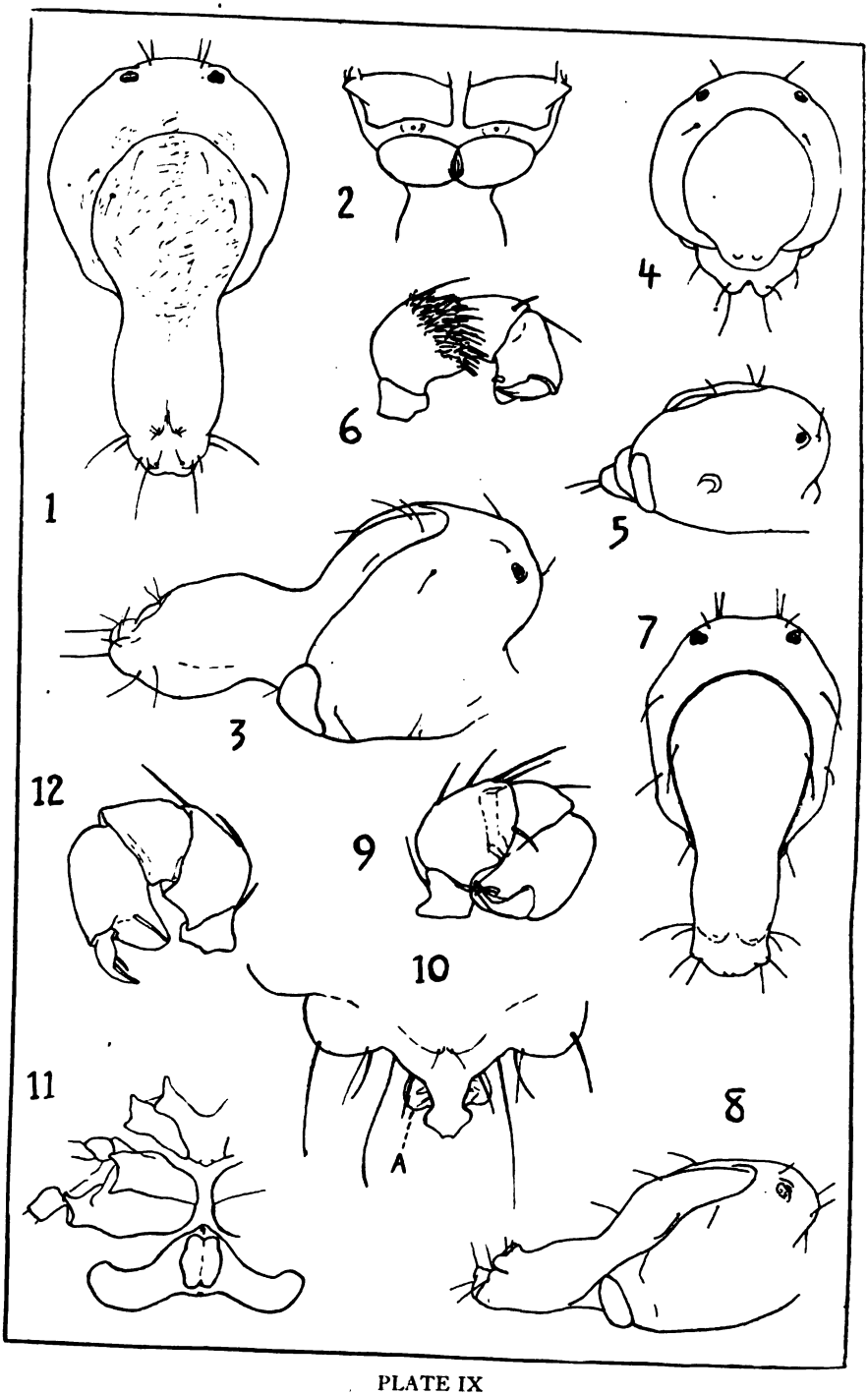

PLATE IX

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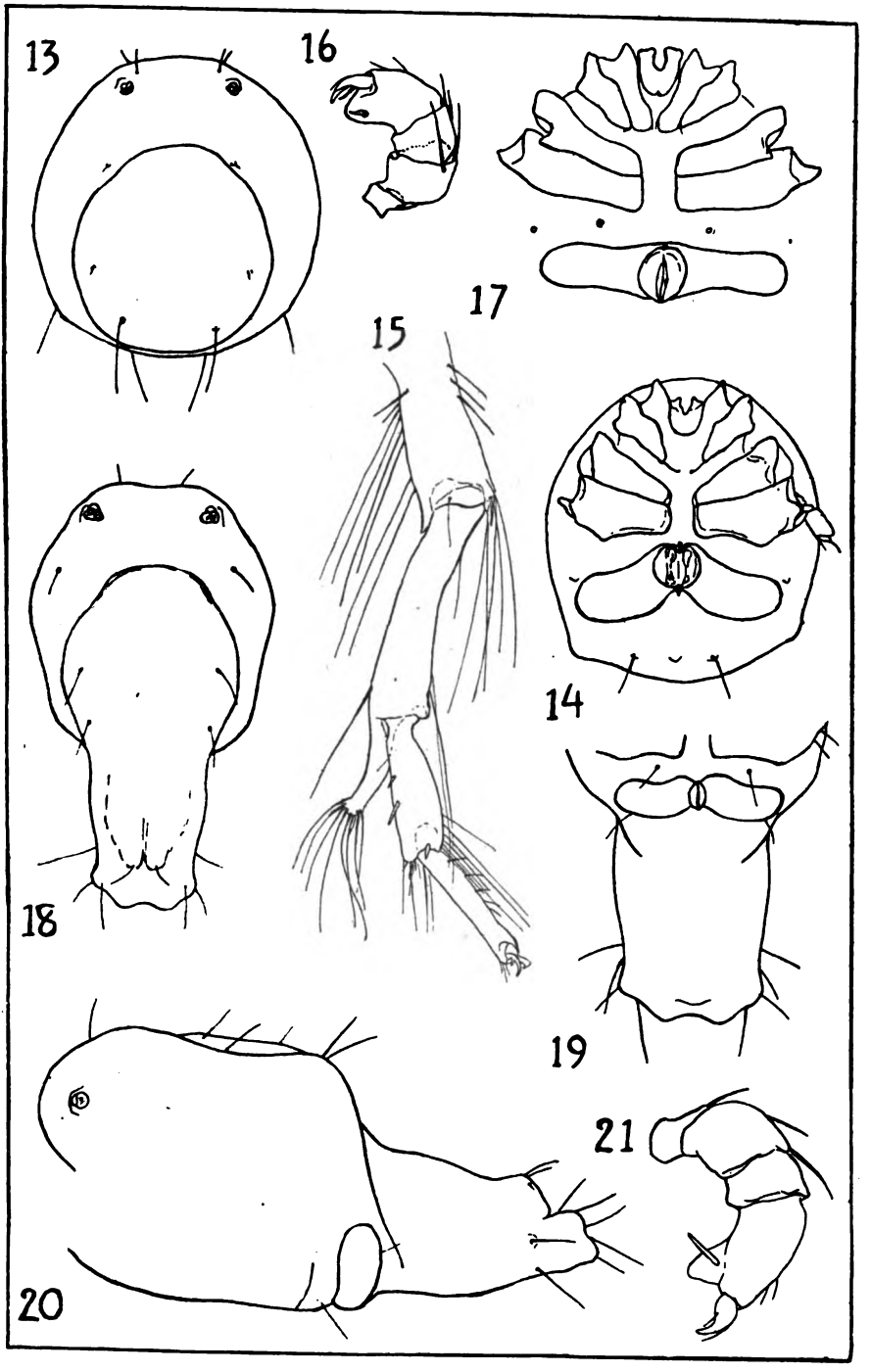

PLATE X

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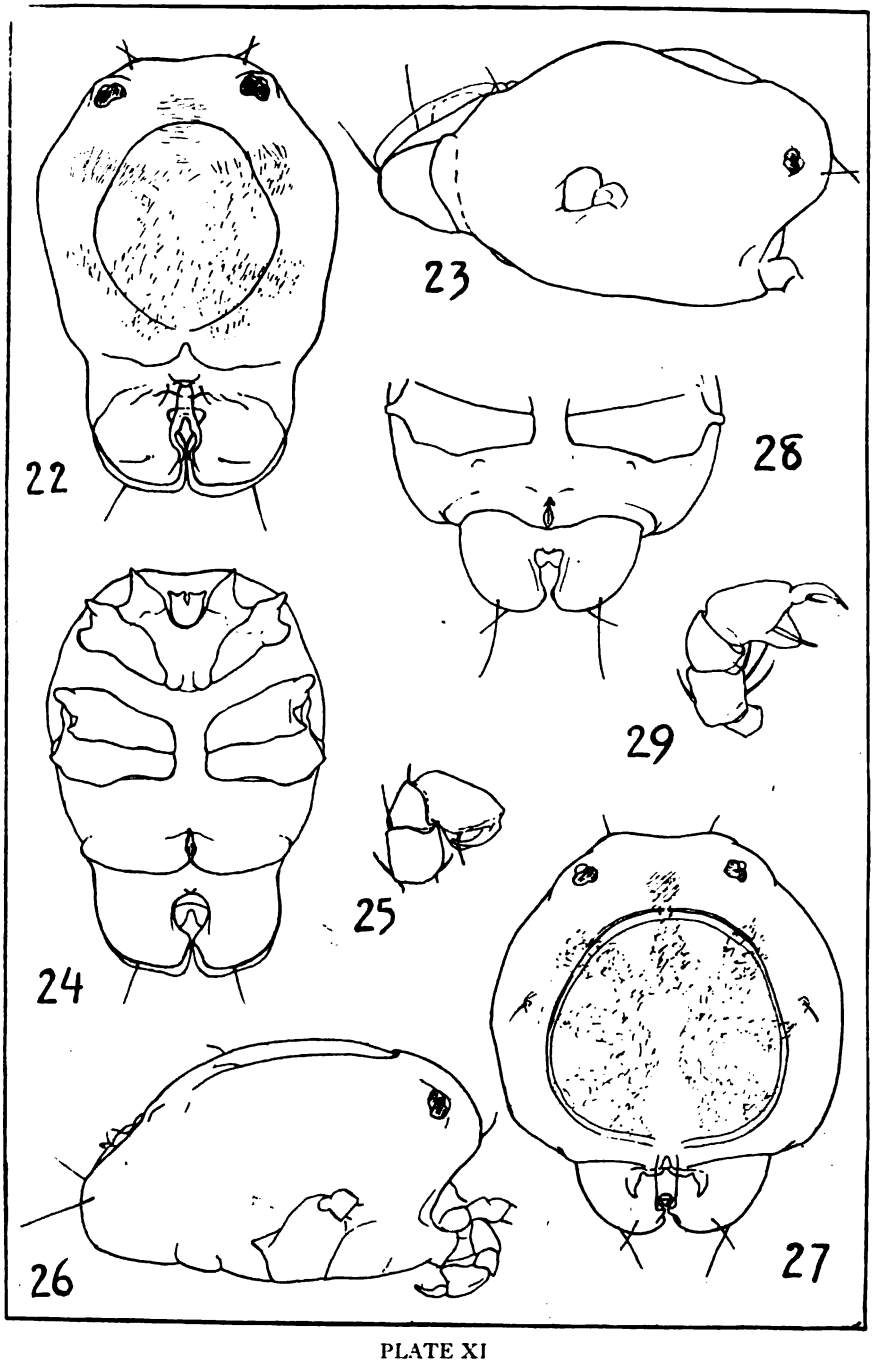

PLATE XI

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SOME WORK ON MARINE PHYTOPLANKTON IN 1919

By
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On September 1, 1919, efforts to use tow nets for quantitative work with phytoplankton in the La Jolla area were abandoned and the resources of the Scripps Institution available for such work were temporarily concentrated upon collection and study of a series of catches made from our pier by the measured water method at intervals of twelve hours. These catches were taken by the simple procedure of dipping water from the surface of the sea at a point about one thousand feet from shore and immediately pouring it through a filtering net made of number 25 bolting silk. This net was made in the form of a funnel into the small end of which a bottle or other receptacle could be tied. After filtration catches were preserved in formalin for quantitative study at convenience.

Quantitative studies consisted in roughly approximate identifications of the species present and in an enumeration of representatives of each found in a certain fractional part of a catch mounted in a Sedgwick-Rafter counting cell. Necessary aids to this work were a Whipple eyepiece micrometer and a mechanical stage. Records were later assembled in the form of tables which were studied with reference to occurrence and prominence of species in the locality at the time and through the period of observation.

It is interesting to note that the locality in which this work was done was much farther south than points at which extensive studies of phytoplankton have been made in Atlantic waters, i. e., it is in about the latitude of Northern Egypt. Furthermore the fact that catches were made regularly and continuously for months at intervals of twelve hours (8 a. m. and 8 p. m.) marks the series as somewhat different from most other groups of catches of marine plankton material.

Surface water temperatures taken at the time of making the catches showed a range from 23° C. in August to 13° C. in December, but the range within the limits of discussion of this paper was from 208° C. in September to 13° C. in December.

Although there are other plants which take some part in synthetic activities in the open sea and although dinoflagellates of many kinds are generally analytic rather than synthetic in character, it has appeared from preliminary studies of marine plankton in the Southern California region that the groups most promising for quantitative study as synthesizing organisms are the diatoms and dinoflagellates, (or at least the armored dinoflagellates). There seems to be no reasonable doubt that these two

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groups of organisms are on account of their small size, large numbers, rapid growth, facile reproduction, wide distribution and cosmopolitan character peculiarly favorable objects for quantitative study especially since they seem to be the most easily and continuously accessible of all marine organisms.

Both groups show rhythms and pulses of production which are more or less evident in each month of the year. Such rhythms and pulses are, however, characterized by changes in prominence of particular species according to season and according to certain other variable conditions.

For convenience in the present discussion a pulse may be defined as a marked increase in numbers of organisms which extends over a period of three or more days before decreasing to or near the numbers found at its beginning. In this four month period there were five such pulses of diatoms and four of dinoflagellates. In both groups they were unevenly distributed in the period. The records show that out of four times possible for coincidence of pulses of the two groups there were two close approximations to coincidence. This, of course, indicates that both groups of organisms are sometimes favored by the same stimuli to production.

But it is true that some other evidence indicates different possibilities, e. g., there were fourteen cases in which a catch of diatoms was more than three times as large as either the catch preceding or the one succeeding it and there were fifteen such cases of dinoflagellate catches. Out of fourteen chances for coincidence of such catches in the two groups only four occurred, a fact which leads one to think they may be to some extent mutually deterrent. This view gains support from the fact that catches distinctly low in numbers as contrasted with those catches nearest them show no coincidence in five chances although we might expect that there would be coincident absence in both groups if both were similarly responsive to changes in local conditions. In view of such considerations one seems to be driven to the provisional assumption that plants in the open sea like those on land may sometimes find such generally favorable conditions that widely different types may live and thrive together without prejudice but that usually some factor has given one form a better opportunity than another which may be used to the detriment or to the complete exclusion of that other.

The above mentioned exhibits of presence and absence are still more suggestive in regard to the perennial assumption that marine organisms are uniformly distributed through considerable areas of marine waters. A catch markedly larger than both of those at twelve hours from it or a catch markedly smaller than both surely indicates that distribution is not uniform in the given area.

Since catches were taken rather early in the day and early in the night the records were examined for evidence of greater productivity in light

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or darkness. In October and November, two months out of the four, about four-fifths of the larger catches were made in the morning and in the other two months there was not much difference. So far as this limited evidence goes it favors the view that growth and reproduction occurs most vigorously at night, as might be expected from our general knowledge of distribution of plant activities in the twenty-four hours.

Although many species of diatoms and dinoflagellates may be found in the Southern California region there are not many which are ever very prominent or numerically important and there are very few which are frequently and continuously thus important. Since most of these can be identified fairly well under ordinary conditions of examination, statistical study is not seriously hampered by the requirements of taxonomy.

For most purposes it is best to study the distribution of diatoms and dinoflagellates as separate groups. Thus considered the following points may be noted concerning diatoms: Some representatives of the group were to be found throughout the period although distribution was very irregular. Large numbers appeared in the last three months of September thus producing an autumnal maximum similar to those noted in European waters.

In connection with this maximum I was interested in noting that for two or three days previous to its inception there had been rather strong and constant currents from the north. I also noticed that large numbers of mackerel came to the vicinity of our pier in the latter part of August and left about the time that the great increase in diatom prduction began. Whether these points were mere coincidences or whether they had significant relationship to increased diatom production, I have no means of knowing

Forty six species of diatoms were recorded in the four months but only twelve of these were readily identified although fourteen were usually approximated, i. e., confusion limited to only one or two other forms. These included most of those of numerical importance. Eleven forms were found to have been represented in the most abundant five in one or more months. Five of these belonged to the genus *Chaetoceras*. They were mostly rather small species and difficult to identify.

Detailed study of the records has clearly shown the important fact that when there is increased production of the most prominent forms there is also increased production of the less prominent forms and an increase in the number of different forms. Such facts naturally lead to the assumption that conditions favorable to high productivity of diatoms in the sea affect a large number of forms in the same way. They also lead to the inference that determination of the species which shall lead in production is largely due to biological factors such as rapid multiplication and vigorous development.

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As to the dinoflagellates I may say that they are usually much fewer in numbers than are the diatoms. Otherwise the general features of their distribution are not greatly different except in the periods of maximum production. In the last four months of 1919 the greatest numbers were produced in November but there had also been some heavy production in August several weeks before the maximum production of diatoms.

Thirty-seven forms of dinoflagellates were recorded eight of which were fairly easy to identify. Usually satisfactory identification of twelve forms could be made and these included most of those showing numerical importance. Six easily identified species were found amongst the five most numerous in one or more of the four months. Two of them belonged to the genus *Ceratium*.

Two of these most prominent species deserve special mention because of their connection with the phenomenon called "red water." *Gonyaulax polyedra* Stein has at various times been mentioned as responsible for extensive areas of "red water" in Southern California which have attracted especial attention because of the bad odor where it was washed upon the beaches and because of the large number of littoral animals killed by it and then stranded upon the beaches. The brownish or reddish color of the water is due merely to the vast numbers of these small organisms present in it. The destruction of littoral animals is usually said to be due to products of decay after death of such quantities of the microscopic organism. But it is possible that the living *Gonyaulax* is also poisonous to animals. In the last three or four years *Prorocentrum micans* Ehr. has been more often detected as a cause of "red water" than has Gonyaulax but no cases have been reported in which littoral animals died as a result of its presence. It is noticeable that in "red water" areas (some of which extend for miles in open water) very few other organisms, large or small, are found amongst those which cause the discoloration.

Several different kinds of dinoflagellates cause the appearance of water called "phosphorescence." More or less glow of this sort may be observed in waters of our section at almost any time of year although not continuously present. At times there are present in the water sufficient numbers of individuals of this sort to cause at night a glowing pathway where fishes stimulate them by swimming through.

A more detailed report of this work in 1919 is awaiting publication in another place. Its conclusions may be briefly stated as follows:

First, the measured water method seems to be by far the best to use for a *standard* method and the surface level to be the best for a *standard* level of collecting for quantitative study. Other methods and other levels should be regarded as special methods more or less supplementary to the standard.

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Second, there is evidence that drift currents have pronounced influence on phytoplankton production at our pier.

Third, large numbers of phytoplankton organisms respond to conditions of production favorable for any one.

Fourth, it seems probable that some of the more prominent forms may be useful as indicators of certain conditions in the ecologic complex.

Lastly, it is evident that the problems of the ecologic complex of the sea are fascinating as well as intricate and baffling and that in many ways good returns are sooner or later to be expected as the results of time and energy expended in study upon them.

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THE ACCESSORY CHROMOSOME OF ANASA TRISTIS AGAIN

By
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During the examination of literature in connection with cytological studies on other Hemiptera I became much interested in the case of Anasa tristis. I was astonished to find a very marked variation in the results of the cytologists who have studied the spermatogenesis of this form. Therefore, when in the summer of 1919 there occurred an excellent opportunity to procure an abundance of material, I decided to make some observations of an independent nature. It would seem not out of place occasionally to examine some of the commonly accepted cases and particularly where, as in this instance there has been a decided disagreement.

Altogether ten investigators have worked on the male germ-cells of Anasa tristis. At one time or another four of this number have been opposed to the now generally accepted view, first stated by E. B. Wilson. Two of these have corrected their former statements and now agree with the latter in his conclusions.

Paulmier ('99), who was the next after Henking ('91) to study the history of the accessory chromosome, decided that the spermatogonial number of chromosomes was twenty-two. He discovered the pair of m-chromosomes in the spermatogonia and described their behavior. He believed these united in synapsis to form a single condensed bivalent chromosome-nucleolus which persisted throughout the growth period and became the small central tetrad of the first maturation division. Furthermore he stated that this tetrad divided equally in the first division but that the products of this division passed undivided to but one of the poles of the second spindle giving ten and eleven chromosomes respectively to the spermatids. He therefore identified the chromosome-nucleolus of the growth period as the microchromosome bivalent and thought this to be identical with the accessory.

Montgomery ('01, '04) followed Paulmier in giving the spermatogonial number of chromosomes as twenty-two. He also regarded the accessory chromosome as being derived by a fusion of the m-chromosomes. A re-examination of his material after the publication of Wilson's second paper on chromosomes led to a change in his statements so that they were then in agreement.

After a very careful study of Paulmier's material as well as his own Wilson came to the following conclusions in a remarkable series of papers ('05, '06, '07, '11); (1) that there are twenty-one and not twenty-two

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chromosomes in the spermatogonia; (2) that these chromosomes exist in pairs and this leaves one without a mate; (3) that this odd one is one of the three largest and is the so-called accessory chromosome; (4) that this chromosome exists as a chromosome-nucleolus throughout the growth period; (5) that the m-chromosomes previously identified by other observers as the accessory have an entirely independent history and divide in both of the maturation divisions; (6) but that the real accessory divides in the first and then passes undivided to one only of the two spermatids derived by division of the secondary spermatocytes, thus giving rise to two kinds of spermatozoa.

Foot and Strobell ('07), using smear methods to the entire exclusion of sections and illustrating only with photo-micrographs, took sharp issue with Wilson. These investigators asserted that Paulmier was right in his spermatogonial count; that the so-called chromosome-nucleolus of the growth period is but "morphologically the equivalent of a nucleolus" or in other words the plasmosome; that there is no odd or accessory chromosome; that what has been called such is but a lagging chromosome which divides in each division as do all the others; that therefore all spermatids receive eleven chromosomes.

This disagreement among cytologists, of course, became a serious matter. Many fundamental facts came into direct question and consequently several people were interested enough to make independent investigation of the conditions.

Closely following the papers of Foot and Strobell there appeared a brief treatment of the question by Lefevre and McGill ('08). Their observations confirmed those of Wilson.

In connection with work on the chromosomes of some of the coreid Hemiptera Morrill ('10) confirmed Wilson's spermatogonial count.

The climax of the researches on the accessory chromosome of Anasa came in 1910 when McClung and Pinney went over the whole matter with great care. Miss Pinney made an entirely independent study and in order to avoid bias or prejudice in the matter refrained from reading any of the accounts published by other investigators until her own conclusions had been reached. McClung studied the original material of Paulmier, Wilson and Lefevre and McGill. Both McClung and Pinney agreed that the spermatogonial number is twenty-one. They further agreed with Wilson that there are ten bivalent chromosome and one, the accessory, which is univalent in the metaphase of the first division. This univalent body exists as a short, heavy thread, a compact mass or finally as a straight longitudinally split rod all through the prophase stages of the first division. It divides as do the others in the first but does not divide in the second mitosis.

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In making this brief and confirmatory study of Anasa tristis[1] I have used the ordinary cytological methods now somewhat standardized. As usual I have found Bouin's and Flemming's fluids very valuable. Perhaps the best preparations have been made with Bouin's. The iron-hæmatoxylin method of staining has again proven the best general stain although I have had difficulty with the domestic preparations

My own conclusions are not enough different from those of Wilson to warrant a lengthy treatment. In fact as regards the important stages the matter might be dismissed by a statement that the facts as I see them are as stated by Wilson, Lefevre and McGill, and McClung and Pinney. In order, however, that my results may be on record and that the constancy of the chromosome relationship within the species may be further evidenced I will state the main facts as I see them.

There are without question twenty-one chromosomes in the normal spermatogonia. I have examined dozens of these cells in the metaphase when the chromosomes are well spread out but I have never found one which clearly showed more than the expected number. The three large bean-shaped chromosomes and two small m-chromosomes are always present together with sixteen others of about equal size.

In the late prophase stages of the first division at least nine typical tetrads can be seen accompanied by what I think are three dyad bodies. I am sure one of these is the accessory. Probably the others are the m-chromosomes but the study of these has not made me entirely sure of this. When the chromosomes become placed in the metaphase plate preparatory to division the accessory usually occupies a position outside of the ring formed by the position of the nine ordinary tetrads. The m-chromosomes, now unquestionably formed into a tetrad, occupy a central position within the ring. All the bodies divide equally in this division. As a result then all secondary spermatocytes possess eleven chromosomes each. When these become arranged in the flat metaphase plate again the ring-like arrangement is succeeded by an irregular placing. In spite of this the accessory can usually be identified. When the second division occurs this accessory does not divide like the rest but goes to one pole undivided thus giving rise to two kinds of spermatids. Lateral and polar views of these stages show without the shadow of doubt that half the spermatids receive ten while the other half get eleven chromosomes.

At this point I will mention a condition observed several times in Anasa and other Hemiptera. Follicle cells in division frequently show double the normal spermatogonial number of chromosomes. How this is brought about and what the fate of the cells involved is I am not able to state.

[1] For the identification of my material I am indebted to Dr. Paul S. Welch of the Univ. of Michigan, Ann Arbor, Mich.

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It is amazing that there should have been so much disagreement in the results of investigations up to this point. As is often the case in the Hemiptera when the preparations are good most of the stages so far outlined stand out with diagrammatic clearness. There should be no further difference of opinion in regard to these matters. Anyone can demonstrate the truth of the statements by using reasonable care in preparation and observation.

When we come to the question of the behavior of the accessory chromosome from the time of the last spermatogonial division down to the late prophases of the first maturation division the facts are more difficult to determine. Those who have worked upon the question of spermatogenesis will agree that it is often difficult to discover just what is going on in the nucleus at this time. However, I believe the main contentions of Wilson can be proven true

In many cells before and during synezesis a compact body can be found just outside of the more or less tangled mass of chromatin threads. Because of its size and appearance I think it reasonable to conclude that it is the accessory chromosome. In every stage following this the same body can be identified and need not be confused with the plasmosome which occurs with it for a large part of the growth period. Thus the accessory maintains its individuality throughout the maturation process.

In conclusion I would say then that I believe the current explanation of the "case of Anasa tristis" as given by text-books o' zoology and genetics based upon the work of Wilson is correct. This classical example may be regarded as a permanent addition to our stock of knowledge. Our interpretations may change, of course, but the facts will stand as Wilson stated them.

The different stages of the spermatogenesis of Anasa are on the whole so clear and beautiful that I can recommend them for class use where it is desired to demonstrate the main facts connected with the behavior of the sex chromosome.

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

THE LITERATURE OF DIATOMS

By
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The literature on the subject of diatoms is both extensive and expensive; many of the books are long out of print; much is scattered through periodicals English and foreign; and there is no recent general hand-book at a moderate price. The nearest approach to such a volume is in German, it is the part on Peridiniales and Bacillariales in Engler and Prantl's Pflanzenfamilien, published at Leipzig in 1896. It is written by F. Schuett, and gives an account of the morphology and biology of diatoms, with a scheme of the genera divided into Centricae and Pennatae, a description of each genus then established, and a drawing of one or more species to illustrate the genus.

The Diatomaceae of the Hull District by Mills and Philip contains several plates covering most of the species commonly found in England. There is also a most instructive paper by Philip in the Transactions of the Hull Scientific and Field Naturalists' Club vol. IV. part iv (1912) p. 205, on Diatoms of the Humber.

For fresh water diatoms Die Süsswasser Flora Deutschlands, Oesterreichs, und der Schweiz, part 10, by H. von Schönfeldt deals with diatoms, it is published by Fischer of Jena, and contains many figures of English diatoms.

The early history of our knowledge of the Diatomaceae is given by Ehrenberg in the introduction to the Bacillaria in his first great work, Die Infusionsthierchen, published in 1838. Kitton in Science Gossip, 1880, pp. 78 and 133, gives a résumé of this introduction, which describes the work of Ehrenberg himself and other diatomists. Kitton also gives an interesting account of the views of Corda (1835) in Science Gossip, 1882, pp. 6, 22. The imperfection of the instruments then available, and the then commonly received belief in the animal nature of diatoms often led to the enunciation of opinions, which now appear ridiculous.

Kuetzing's Kieselschaligen Bacillarien, (1844), also contains an historical preface. This was translated by Professor H. L. Smith of Geneva, U. S. A. in nos. 2 and 4 vol. II of The Lens. A résumé of this translation with comments of his own was given by Kitton in Science Gossip, 1874, pp. 2, 25, 149.

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Ehrenberg may be regarded as the father of diatomology, he was able to command a vast quantity of material; and although many of his views are now known to be erroneous, and many of his figures are incorrect or insufficient owing to the imperfect objectives he used, and to the want of sufficient magnification, his labors will always be of great value to the diatomist. About one third of the genera now recognized were founded by him. After him Greville and Grunow are the most prolific creators of genera, accounting for about another third between them.

Diatom literature may be taken as starting with Agardh's Systema Algarum in 1824, and Ehrenberg's early papers on the Infusoria, in which he included diatoms, published in 1829-1832. Kuetzing's Synopsis Diatomearum followed in 1834, and in 1838 Ehrenberg brought out his great work on Infusorial Animalcules, Die Infusionsthierchen, nine of the 64 plates being devoted to diatoms.

Kuetzing's second work, Die Kieselschaligen Bacillarien, 30 pl. was published in 1844; the following year saw the first edition of Pritchard's Infusoria, further editions were issued in 1852, 1861, and 1864 The part on the Diatomaceae was written by Ralfs, and plates iv to xvii give figures of diatoms. This is, perhaps, the book on diatoms most readily accessible to English students, and though portions of it are out of date or incorrect, it forms a very useful introduction to the subject.

Rabenhorst's Süsswasser Diatomaceen, 10 pl., appeared in 1853. Owing to the imperfection of optical apparatus many of the figures in these earlier works are wanting in detail. It is therefore often difficult to determine the identity of the species named and described; yet it is wonderful how much was seen and accurately recorded with instruments that would now be despised and rejected.

The last half of the nineteenth century was a period of great activity in this branch of research. From the year 1853 to 1866 the Transactions and Journal of the Microscopical Society, and the Transactions of the Royal Society of Edinburgh contain a number of papers by Brightwell, Gregory, Greville, Lauder, Roper, Walker-Arnott, Wallich and others, many of which are beautifully illustrated by Tuffen West. William Smith's Synopsis of British Diatoms, containing 69 plates by the same artist was published in 1853-1856; Gregory's Diatoms of the Clyde appeared in 1857 (Proc. Royal Soc. Edinburgh, vol. xxi, p 473). The figures were drawn by Greville.

Ehrenberg's colossal Mikrogeologie appeared in 1856. Besides his two large books he wrote numerous papers on diatoms in the Transactions of the Berlin Academy of Science. Naturally in the course of the progress of knowledge, and with the improvement of instruments, and the increase of material, the early conceptions of many genera and species have been modified, and relations have been acknowledged between forms at first

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sight widely differing. The Micrographic Dictionary first appeared in 1854, with enlarged editions in 1859, 1872, and 1882. It contains several plates of diatoms.

Greville's fine series of 20 papers on New and Rare Diatoms, mostly from Trinidad, Barbadoes, Moron, Monterey, and Ceylon, appeared in the T. M. S. and Q. J. M. S. from 1861 to 1866, with monographs on Asterolampra, Campylodiscus, and Auliscus. He also wrote on Diatoms from Hongkong (A. M. N. H. 1865), the South Pacific (Edin. N. P. J. 1863) and The Tropics and Southern Hemisphere, (T. B. S. Edin. and Edin. N. P. J. 1865, 1866). A collection of poor photographic reproductions of 81 plates mostly Greville's from T. M. S., etc., compiled by Moebius was published in New York. It is sometimes on the market.

In America J. W. Bailey wrote on American Bacillaria, and published several papers between 1841 and 1860. L. W. Bailey also wrote on the subject, and numerous papers by Dr. A. M. Edwards appeared in various periodicals between 1859 and 1877; his sketch of the Natural History of the Diatomacea is dated 1874; (cf. Bull. Torr. Bot. Club, 1877 p. 34). Lewis wrote in 1861 on New and Rare Diatoms, and in 1865 on White Mountain Diatoms.

Cleve, a Swede, began to write in 1864, and published various papers and books, many of them in English, on Diatoms from Spitzbergen, the Sea of Java, West Indian Archipelago, Greenland and Argentina, and Finland; also on Arctic Diatoms, and New and Little Known Diatoms, and on the diatoms found by the Vega Expedition, and later, several papers on Plankton Diatoms. His great work is his Synopsis of the Naviculoid Diatoms published in 1904, 1905. In this last book he proposes a rearrangement of the Naviculae and the related genera setting up a number of new genera, which have not been universally accepted; although many of them are recognized as useful subdivisions of the older genera; some writers, however, accept his proposals en masse.

Grunow, an Austrian, wrote from 1860 to 1890; his papers on New Diatoms, and on Austrian Diatoms (1860, 1862, 1863, and 1882 and 1883) are important contributions to diatom classification; and much of his work is embodied in Van Heurck's Synopsis of the Diatoms of Belgium, and in Schmidt's Atlas. His account of the diatoms of the Novara Expedition is dated 1867; the same year saw his paper on the diatoms of the Sargasso Sea of Honduras, an abstract of this is given in M. M. J. 1877, p. 165. He also wrote on Caspian diatoms, and conjointly with Cleve a book on Arctic diatoms, followed by his Diatoms of Franz Josef Land.

Kitton wrote from 1868 to 1884 a number of articles, many of them in Science Gossip, a magazine which during the years 1867 to 1877 printed several useful papers on the subject of diatoms, including some by Kitton on North American deposits. Kitton translated some of Grunow's

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papers for various English periodicals; among these were the Novara diatoms, which appeared in Grevillea in 1872.

Harting's Banda See, Janisch on Guano diatoms, Janisch and Rabenhorst on the Marine Diatoms of Honduras, Heiberg's Danish Diatoms, and Schumann's Prussian Diatoms belong to the early sixties.

In the seventies followed De Brébisson on Diatoms contained in Corsican Moss. He had previously written on diatoms from the Cherbourg littoral, and wrote other papers.

Donkin published only three parts, (12 plates), of his Natural History of the British Diatomaceæ. About the same time appeared O'Meara on Irish Diatomaceæ (poor plates) and on diatoms from Kerguelen's Land; and Petit on Table Bay, Campbell Island, Cape Horn, etc. In 1872 Pfitzer wrote on the structure and development of diatoms; an abstract of this important paper is given by O'Meara in Q. J. M. S. 1872, p. 240. The Lens, published in Chicago, only lived two years, 1872, 1873. It contains valuable articles by Professor H. L. Smith and other American diatomists; among these is the professor's Classification of diatoms into Rhaphidieæ, Pseudo-Rhaphidieæ, and Crypto-Rhaphidieæ, which was followed by Van Heurck and De Toni, and by most diatomists until the present division into Centricæ and Pennatæ, (a modification of it) appeared. Many French diatomists follow Pfitzer's division into Placochromaticæ and Coccochromaticæ, which is based upon the nature of the endochrome.

In 1874, Adolf Schmidt, a canon of Aschersleben, commenced his splendid work, Atlas der Diatomaceenkunde. This is published in parts of four plates issued at irregular intervals. An index to the first four series, 240 plates, has been published. Up to August 1914 parts 1 to 79, containing plates 1 to 316 had appeared: parts 80-83 have been published since the war. Adolf Schmidt died in 1901, but the Atlas has been continued by his son and by Fricke, Heiden, and Hustedt, with the assistance of Cleve, Grunow, and other diatomists.

J. Brun of Geneva entered the field in 1880 with his Diatomées des Alpes et du Jura; following in 1889 with Diatomées Fossilies du Japon written in collaboration with Tempère; and in 1891 with Diatomées, Espèces Nouvelles. The plates in the two last are excellent: the first commences with an important sketch of the natural history of diatoms.

In 1886 Count Castracane's report on the diatoms found by H. M. S. Challenger was issued by the British Government. This contains 30 plates and valuable notes. Peragallo's Villefranche appeared in 1888 and Wolle's Diatomaceæ of North America (112 plates) was published in 1890 The plates are mostly copied from other authors, and are far behind the originals in execution; but the book is useful for reference, and is generally procurable at a moderate price, considering its size.

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Le Diatomiste edited by Brun, a periodical devoted to the study of diatoms, only lived from 1890 to 1896. It contains many notable contributions including monographs on Pleurosigma and Rhizosolenia by Peragallo, on Entogonia by Bergon, and on the miocene diatoms of Barbadoes by Brun and Barbo.

Le Micrographe Préparateur under the editorship of J. Tempère ran from 1893 to 1906. It contains valuable papers on the structure and reproduction of diatoms, on the movement of diatoms, and on mounting and cleaning. But the most important is Les Diatomées Marines de France, of which some 115 plates were published in this periodical; the complete work, 137 plates, was separately published in 1908.

Leuduger-Fortmorel between 1879 and 1898 published books on the diatoms of the north coast of France, Ceylon, Malaysia, and West Africa.

In 1886-1887 in the Journal of the Quekett Microscopical Club appeared the well known paper on the Oamăru deposit in New Zealand by Grove and Sturt; also in 1886 Pantocsek published the first volume of his great work on the fossil diatoms of Hungary. The second volume appeared in 1889, and the third in 1892. The whole contains 102 plates. In the third volume are many new forms from Kusnetzk in Russia, and from fossil deposits in Japan. A second edition was issued in 1903-1905. Pantocsek has also written on the diatoms of Lake Balaton (Platten See), and of Kertsch, and of Szliacs in Hungary, (1902, 3). About this time Walker and Chase, and Kain and Schultze wrote on diatoms in America, the latter pair bringing to notice the interesting deposit of Atlantic City.

Van Heurck's Synopsis des Diatomées de Belgique, (1880-1885) contains 141 plates, and is a work of the greatest value. His Treatise on Diatoms translated into English by Dr. Wynne Baxter, was published in 1896 before the original; it contains 35 plates illustrating all the species found in the North Sea and the neighboring countries; and in the text are descriptions with typical figures of all the genera known at the time of publication, some 193 in number. Otto Witt in 1885 gave an account of the diatoms from the marine deposits of Ananino and Archangelsk in the province of Simbirsk in the interior of Russia.

Rattray's monographs on Aulacodiscus (J. R. M. S. 1888); Auliscus (J. R. M. S. 1888); Actinocyclus (J. Q. M. C. 1890); and Coscinodiscus (Proc. Royal Soc. Edin. 1890) are standard authorities.

About the same time appeared Pelletan's Les Diatomées, he was assisted by Deby, Petit and Peragallo. The account of the natural history of diatoms is good, and there are numerous illustrations of the most common species and genera. Truan and Witt's book on the fossil deposit of Jérémie in Haiti is dated 1888. Another work of great utility, though it is not illustrated, is Les Diatomées du Mond Entier, issued by Tempère and Peragallo as a companion to the series of 625 slides from various locali-

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ties distributed by them. It gives lists of the diatoms found by them on the slides, and is a valuable aid to identification. A second series of 1000 slides is accompanied by a volume bearing the same title, the second edition of this is dated 1915; it is of course more comprehensive.

Deby's monograph on Campylodiscus appeared in 1891; with three or four exceptions the figures are copied from other works. About the same time a portion of Janisch's report on the diatoms of the Gazelle Expedition was privately distributed to certain favoured diatomists without text or list of contents. This work has never been completed; out of 22 plates numbers 7, 8, 10, 12, 13, 14, 17, and 18 are wanting. Many of the specimens figured are reproduced in Schmidt's Atlas.

In 1891 to 1894 De Toni, a professor of the University of Padua, published his monumental work, Sylloge Bacillariearum as part of his Sylloge Algarum. This book has no illustrations, but it contains in Latin descriptions of all the genera and species described or named at the date of publication, with their synonyms and varieties, and references to all published figures and drawings. Nearly 6000 species are admitted. The book also contains a bibliography to date of the literature of diatoms by Deby.

Frère Héribaud of the college of Clermont-Ferrand wrote in 1893 on the diatoms of Auvergne. The volume commences with a short, but very clear, instructive, and succinct account of the subject, forming an admirable introduction to the study of diatoms. Between 1902 and 1908 he published four memoirs on the fossil diatoms of Auvergne.

In 1896 Schuett wrote the part on diatoms in Engler and Prantl's Pflanzenfamilien, (lieferung 143-145). This book, with its one or more typical figures of each genus, and its account of the morphology and biology of diatoms, forms a most useful handbook at a moderate price. It gives a description of all known genera classified as Centricæ and Pennatæ, the division now generally adopted; the Pennatæ are since 1902 subdivided into Mobiles and Immobiles, the Centricæ are all Immobiles. This last distinction is due to Mereschkowsky, a Russian diatomist: (Script. Bot. Hort. Imp. Petrop. 1902) and A. M. N. H. 1902, p. 65).

Karsten's book, Die Diatomeen der Kieler Bucht, 1899, is praised by Cleve and Mereschkowsky as a vade mecum for students of living diatoms.

In the present century we have Dippel's book on the Rhine and Maine districts, 1905; and a most useful and instructive report by Mann on the diatoms found in the Pacific by the U. S. ship Albatross, published by the Smithsonian Institute in 1907. Von Schonfeldt's Diatomacæ Germaniæ was published in the same year.

Peragallo's magnificent work Les Diatomées Marines de France, (13 plates, 1897-1908), and Meister's Kieselalgen der Schweiz, (48 plates, 1912), contain splendid figures, and include most of the English forms.

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La Diatomologia Española by Azpeitia, (1911, 12 plates), treats of various Spanish deposits, including Moron. This establishes two quite new genera, Dossetia and Secallia.

I must not omit mention of three important papers by Laubeg, on the Palæobotany of France, (Soc. Bot. de France. Mém. XV. Jan. 1910): the Study of Sedimentary Deposits of Diatoms (Bull. des Services de la Cante Géologique de France, Mém. 125, 1910): and on Diatoms, their Deposits and Uses, (Revue Générale des Sciences, 1911).

In Nuova Notarisia professor Achilli Forti has written several papers, with photographic illustrations; on Bergonzano and Marmorito deposits in 1908 and 1914; and a monograph on the genus Pyxilla in 1909. His paper on the classification of diatoms as Mobiles and Immobiles in 1912 was anticipated by Mereschkowsky in 1902, (Script. Bot. Hort. Imp. Petrop. Fasc. xviii. p. 96.), as I have already pointed out. Mereschkowsky has also written other papers on diatoms, and has suggested the formation of various new genera, some for new forms, and some for species already described.

West's Algæ (Camb. Univ. Press, 1916) devotes 43 pages to Diatoms, and compresses much information into that space. Boyer's Diatomaceæ of Philadelphia, 40 plates, bears date 1916: it contains a useful introduction and fine illustrations.

Considerable attention has been given during the last thirty years to the Plankton diatoms. Cleve in 1889 wrote on Pelagic diatoms from the Kattegat, and followed this by papers on diatoms from Baffin's Bay and Davis' Straits (1894), Phytoplankton of the North Atlantic (1897), Diatoms of the Jackson-Harmsworth Expedition (1898), North Sea and English Channel (1900), Swedish Expedition to Greenland (1900), and Plankton of the South Atlantic (1900). And Oestrup wrote on the Marine diatoms of East Greenland (1895, 6 plates).

Van Heurck in 1909 wrote on the diatoms found during the voyage of S. Y. Belgica in 1897-1899 in the Antarctic Ocean, (13 plates).

Karsten wrote the account of the diatoms found during the German Deep Sea Expedition in 1898, 1899. This is a costly and magnificent work in three parts, on the Antarctic, Atlantic, and Indian Phytoplankton, with splendid plates. Other writers on the subject are Ostenfeldt, Aurivillius, Hensen, Jörgensen and Lemmermann.

The Belgian Museum of Natural History has published Microplankton de la Mer Flamande, by Meunier, (Tome VII. Fasc. 2, 3. 14 plates. 1913, 1915), Nordisches Plankton, Botanischer Teil, by Gran, 1905, contains about 180 figures of interesting and new forms. Gran also wrote several other papers on Arctic Diatoms and Plankton.

I must also note Mangin's paper on the Study of Plankton in Annales des Sciences Naturelles, 1908, pp. 177-219; and Bachmann on the Phyto-

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plankton on Fresh Water, with special reference to the Lake of Lucerne, (1911, pub. Jena). The report of the Imperial Fisheries Institute of Japan for 1911 contains 6 plates of littoral diatoms of Japan.

It is impossible in the space of a few pages to note all the contributions to the knowledge of the structure and history of diatoms, even to name more than some of the best known writers on the subject such as Nelson, Morland, Lauby, Cox, Brightwell, Roper, Wallich, the two Müllers, Butcher, O'Donohoe, and Murray.

THURGARTON, BOURNEMOUTH.

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A COMPENDIUM OF THE HOSTS OF ANIMAL PARASITES CONTAINED IN WARD AND WHIPPLE'S FRESH-WATER BIOLOGY

COMPILED BY
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University of Illinois

Ward and Whipple's Fresh-water Biology contains by far the most comprehensive treatment of the animal parasites of the North American fauna that has ever been published. The chapters dealing with the parasitic worms, represent contributions, on the part of Professor Henry B. Ward, not only in a compilation of results of almost innumerable researches of varying magnitude but also in the inclusion of extensive data based upon previously unpublished records. Much of this information of especial interest to parasitologists and to field zoologists is not available for ready reference because the names of hosts mentioned in the text are not included in the general index of the book.

For personal use the present writer prepared a compendium of the hosts mentioned in the Fresh-water Biology. This proved so valuable an aid and received such favorable comment from workers to whom the manuscript was shown that it was considered desirable to put it into a form in which it could be generally available.

The authors of the Fresh-water Biology have made no attempt to include complete check lists of the hosts in the chapters dealing with parasitic forms, yet in many instances references are inclusive enough to be of great value as a point of departure in determining the recorded parasitic fauna of any given host animal. In using this compendium it should be recalled that in many instances only one or a few typical species are listed for each genus and even the hosts of such species as are cited do not constitute full check lists. Doubtless there have been numerous erroneous determinations of hosts in the works from which the host lists have been assembled but host names have been quoted directly as they stand in the original citations without attempt at verification. As a result, some of the names of hosts current in the older literature appear along with the valid names of the same species in this compendium.

In many instances where there seems to be no fixed specificity of hosts, as well as in the discussion of families and genera of parasites, group names such as 'fish,' 'birds,' or 'water birds,' are used frequently.

In the chapters dealing with Protozoa the parasitic forms have not received the attention of the writers, consequently the great group of Sporozoa and all other parasitic protozoans have received no treatment in this compendium.

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To facilitate locations of words on the page in referring back to the text, specific, generic, vernacular, or group names have been used in the compendium as they stand in the text reference. In a few instances cross references have been inserted between vernacular and scientific names, but in such instances the page references to the two names have not been assembled. This is due to the belief that the inconvenience of cross citation is less than the confusion resulting from the necessity of visualizing both vernacular and scientific names while scanning the printed page in search for a given reference.

A host name cited under a given page reference may appear more than once on that page.

A direct means of determining the groups of parasites listed for any given host without necessity of referring back to the text is afforded by reference to the following list of page inclusions for the various groups containing parasitic forms:

Trematoda	374-424
Cestoda	429-451
Nematoda	520-535
Gordiacea	537-542
Acanthocephala	545-551
Rotatoria'	553-620
Discodrilidæ	644
Hirudinea	646-660
Copepoda	782-788
Malacostraca	841-850
Hydracarina	851-874

MAMMALIA

'mammals' 390, 404, 440, 441, 442, 444, 447, 522, 549
beaver 386
cat 390, 393, 447
cattle 389, 409
Didelphis virginiana 410
dog 390, 523
Lepus 409
man 389, 409, 432, 433, 434, 521, 522, 523, 534, 656
mink 522, 523, 530
muskrat 383, 386, 391, 404, 447, 451, 522, 534, 535
Mustelidae 522
otter 522, 523
pig 390
seals 433, 523, 549
sheep 389, 408
skunk 522
wapati 389
weasel 522
whales 433
wolf 523

AVES

'bird' 402, 404, 409
'birds' 440, 441, 442, 443, 444, 446, 447, 448, 526, 549, 550
Anas platyrhynchos 388
Anseriformes 449, 548
Ardea herodias 410, 524, 527, 532
Ardea minor 391
bittern 532
Botaurus lentigenosus 550, 551
Botaurus minor 526
canvas back 442
chicken 391, 402, 446
coot 443

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cormorant 533
crane 444
crow ('fish') 446
duck 411, 441, 442, 447
egret (see Herodias)
eider duck 549
Gallinago wilsoni 382
Gavia immer 391 (see loon)
geese 442, 447
grebe 440, 448
grebe (horned) 448, 449
gull 440, 442, 446 (see Larus)
Herodias egretta 549
'heron' 408, 440, 444, 445, 532
heron (green) 444
heron (little blue) 444
herring gull 408
'ibis' 448
kingfisher 525
Larus philadelphia 391
loon 440 (see Gavia)
pelican (brown) 533
pelican (white) 433, 533
pintail-duck 442
Plotus anhinga 524 (see water-turkey)
Porzana carolina 549
scoter 442
scoter (American) 449
scoter (black) 391
shore birds 442, 444, 445
snipe (grey) 382
Somateria dresseri 549
sparrow (English) 384
spoonbill 444
spoonbill (roseate) 445
stilt (blacknecked) 447, 448
waterbirds 401, 431, 432, 442, 443, 446, 447, 548
water turkey 523, 533 (see Plotus)
wood-ibis 531

REPTILIA

alligator 382, 408
Alligator lucius 410
Alligator mississippiensis 391, 530, 531, 532
Amyda 402
Ancistrodon piscivorus 439
Aromochelys carinatus 376
Aromochelys odoratus 376, 377, 397
Aspidonectes 402
Bascanion constrictor 405
Chameleon 400
Chelonura serpentina 524
Chelydra serpentina 376, 384, 387, 396, 529
Chrysemys marginata 377, 385, 394
Chrysemys picta 394
Cinosternum pennsylvanicum 376
Cistudo carolina 522, 524, 533
'Emys' 546
Emys guttata 529
Emys scripta 529
Emys serrata 524, 533
garter snake 407
Heterodon platyrhinus 405, 406, 407
Malacoclemmys geographicus 546
Malacoclemmys lesueurii 378, 380
Nanemys guttata 528
Natrix rhombifer 407, 439
Pseudemys 387
Pseudemys elegans 546
Pseudemys scripta 377
snakes 405, 438
terrapin (common, food-) 376, 386, 531
Trionyx ferox 378
Tropidonotus rhombifer 406
Tropidonotus sipedon 406, 526
turtles 385, 433, 526, 652

AMPHIBIA

amphibians 398, 399, 404, 408, 434, 438, 449, 522, 547
Amblystoma 399
Amblystoma mexicanum 528
Amblystoma tigrinum 438
Anura 400, 403
Bufo 399
Bufo americana 522, 533
Bufo lentigenosus 521
Cryptobranchus alleghaniensis 525
Diemyctylus viridescens 547
frog 382, 399, 400, 402, 404, 387, 408, 411, 652, 654
Necturus lateralis 379
Necturus maculosus 439
Rana catesbiana 408
Rana halecina 531
Rana pipiens 410, 524, 530
salamander 382, 399
Salamandra rubra 533
Siredon mexicanus 528, 533

Digitized by Google

PISCES

fishes 382, 398, 401, 408, 411, 433, 434, 523, 524, 528, 529, 547, 551, 652, 653, 654, 655
Acipenser (European) 392
Acipenser oxyrhynchus 433
Acipenser rubicundus 378, 392, 396
Acipenser sturio 374
Ambloplites rupestris 401, 436, 548, 785
Ameiurus nebulosus 399
Amia calva 392, 401, 432, 435, 436, 526, 548, 787
Anguilla vulgaris 435
Anguilla chrysypa 401, 435, 524, 546
Aplidonotus grunniens 381, 395
black bass 375, 379, 392, 395, 408, 524, 534
white bass 528, 529
blue-gill 408, 411
Boleosoma nigrum 379
bull-head 382, 408, 439
carp (German) 529
catfishes 786
cat (channel) 439
Catostomus commersonii 787
Catostomus teres 375
chub 431
Coregonus nigripinnis 437
Coregonus prognathus 437
Coregonus artedi 437
crappie (black) 528, 529
Cristivomer namaycush 437
Cyprinidae 408, 430
dace 395
dace (horned) 411
darter 395
Dorosoma cepedianum 545, 547
Eromyzon sucetta oblongus 787
Esox lucius 392, 399, 401, 437, 546
Esox reticulatus 392, 437, 524, 529 (see Lucius)
Fundulus 655
Fundulus ocellaris 784
Gasterosteus 432
herring (lake) 527
Ictalurus punctatus 395, 401
Lepomis pallidus 785
Lepisosteus osseus 788
Lepisosteus platostomus 435, 436
Lepisosteus tristoechus 788
Lota lota 392
Lucioperca 392
Lucius masquinongy 787
Lucius reticulatus 788 (see Esox)
Micropterus dolomieu 374, 392, 398, 401, 436, 548, 785
Micropterus salmoides 392, 400, 436, 546
minnow 379, 395, 398, 408, 411
minnow (red-finned) 395
Moxostoma macrolepidotum 394
Perca flavescens 401, 524
perch 379, 395, 396, 399, 408, 411
pickerel 788
pike 408, 411, 521
pike (wall-eyed) 530
Ptychochelius oregonensis 424
pumpkinseed 395, 411
rays 434
Roccus lineatus 375, 548, 785
rockbass 375, 379, 392, 395, 398, 408, 411
Salmo sebago 434, 437
salmonid fish 527
Salvelinus namaycush 392
shad 533
sharks 434
sheepshead 528
Siluridæ 439
Stizostedion canadense 788
Stizostedion vitreum 401
sturgeon 395
sturgeon (lake) 530
sucker 395, 431, 787
sunfish 375, 395, 411
teleosts 431, 524
trout 408, 431, 433, 550
trout (lake) 374
trout (Great Lake) 431, 527, 547, 548
whitefish 432, 521, 527, 547

INSECTA

insect 534, 851
insect larvæ 528
Achaeta abbreviata 539
Acrididæ 538, 540
Blasturus cupidus 395
Blattidæ 538
Gryllus (see Achæta)
Hexagenia 395
Locustidæ 540
mayfly 395
Neobius fasciatus 539

CRUSTACEA

crustacea 528, 534
Apus 411

Digitized by Google

Copepoda 451, 523
crawfish 395, 401, 644
Cyclopidæ 442
Decapoda 842
Diaptomus 442
Isopoda 547
Ostracoda 451, 523
Palaemonetes 842
Palaemon 842

GASTROPODA

snails 409, 415, 451
Campeloma decisum 416, 419, 420
Gasteropoda 417
Goniobasis virginiana 416
Helix albolabris 423
Helix alternata 423, 531
Helix arborea 411
Lymnaea 419
Lymnaea catascopium 411
Limnaea humilis 389
Lymnaea proxima 412, 417, 418, 419
Lymnaea reflexa 415, 422
Lymnaea stagnalis 411
Physa anatina 417
Physa gyrina 412, 417, 419, 420, 423, 424
Physa heterostropha 411, 413
Planorbis parvus 413, 415
Planorbis trivolvis 413, 417, 420, 421
Pleurocera elevatum 416
slugs 451
Succinea 423
Succinea ovalis 409

LAMELLIBRANCHIA

Anodonta 379, 380
Fresh-water mussels 423, 851, 872
Unionidæ 421

ANNELIDA

annelids 451, 521
earthworm 430
Lumbriculus 451
Tubificidæ 430

ROTATORIA

rotifers 554
Albertia 589
Pleurotrocha 589

COELENTERATA

Hydra 287, 291

PORIFERA

freshwater sponges 856

PROTOZOA

colonial protozoa 856

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THE ENDOCRINES, BY SAMUEL WYLLIS BANDLER, M. D., W. B. Saunders Co., Philadelphia and London, 1920. 486 pp.; price, cloth, $7.00 net.

REVIEWED BY
T. W. GALLOWAY

In the sudden enthusiasm generated by new discoveries that are clearly important, we human beings are reasonably sure to be swept into fads and over-emphasis. Both our generalizations in scientific philosophy and the more practical applications of them to the treatment of human ailments are full of illustrations of the momentum of credulity. The student of the history of science will recognize instances under the terms,—"natural selection," "adaptation," "eugenics," "eye strain," "uric acid," and the like, in which truth has been over-pressed.

It would be a miracle under the circumstances if the thoroughly admitted omnipresence of endocrine secretions in the blood, produced by all sorts of normal and abnormal groups of cells, should not congest human traffic over the same course, and lead the willing student of the subject into statements which are not now established. For example, the present author asserts that they (the endocrines) are the underlying factors in heredity;" and, more specifically, "The differences between animals of various species (and among individuals of the same species) are due to the ductless glands," and more of like tenor. While it may well be true that the hereditary structural elements in the germ cells are influenced by the internal secretions of the soma in which the germ cells lie, and both soma and germ cells are profoundly influenced in their later development by their mutual secretions, such over-sweeping statements of causation are not altogether impressive. It is a bit like saying that the environment is responsible for heredity! which is doubtless true. In spite of this over-enthusiasm, the body of the book contains a mass of most interesting statements supported by many cases,—with history, symptoms and experimental therapy given,—as well as by inference. The author is a gynecologist and naturally stresses the hormones linked most closely with the functions of sex development, reproduction, pregnancy, parturition, and with the emotions conne ted with these.

The author with commendable boldness even undertakes to redeem some territory from the deluge of Freudian interpretation, by way of endocrine action. He makes a strong case for believing that many of the complexes, hysterias, bad orientations, and phobias which the psychoanalysts accredit to mental conflicts are in reality due to a poor balance in the endocrine system.

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Rather unfortunately, because the author does not seem to have a large first-hand knowledge of investigation on inheritance, the book is introduced by a somewhat rambling and inconsequential jumble of illustrations and exhortations which is rather euphemistically labeled "Environment and Heredity,"—presumably because these terms combined make room for about all that can be said on any subject. An analogous looseness and lack of system in arrangement mars the treatment at many points and leads to much unnecessary repetition, and to some seeming conflicts. This is particularly illustrated in comparing chapter 3, The Introduction to the Story of the Endocrines; chapter 4, Internal Secretions, and chapter 18, The Balance between the Endocrines. The total is something like what one might use in a series of lectures on the subject to a class in which inattention or lack of preparation would make much restatement seem necessary, rather than what one expects in a scientific book.

Aside from those mentioned, the following are the principal chapter headings: Environment and Heredity; The Endocrines in Gynecology; Hypergenitalism and Hypogenitalism; Skin Affections and Internal Secretions; Puberty and Climacterium; The "Higher up" Theory of Sterility in Women; Pregnancy, Labor and Placental Gland; Constitutional Dysmenorrhea; Instincts and Emotions; Mental and Nervous Defects; Mental Deficiency and Criminality; Neuroses and Psychoses; Phobias; The Autonomic Nervous System; Therapeutic Suggestions concerning Endocrines; and several chapters dealing with histories, symptoms, clinics and cases.

Possibly an illustration will aid in making vivid the author's repeatedly emphasized point of the interdependence of the internal secretions. In respect to lactation after labor, the following facts are significant:—The mammary glands are developed at puberty by the interaction of secretions from the ripening ovaries, the posterior hypophysis, the thyroid and the adrenals. A secretion from the ovaries or from the endometrium causes them to swell at menstruation. Placental secretions during pregnancy produce hypertrophy and differentiation of tissue at that time, and seem to inhibit the action of the above mentioned secretions. Milk itself will not form however until this placental secretion is removed or inhibited. After birth the ovaries, posterior hypophysis and other endocrines resume sway and stimulate degenerative process by which milk is formed.

Assuming that feminine metabolism and emotional states are more variable and instable than the masculine, the explanation by the endocrinologist would include such facts as these:—men and women alike possess these numerous glands and with all degrees of original potency; all these glands are normally pouring their secretions into the blood constantly; the secretion of each gland modifies those of certain, if not all the other glands, as well as general metabolic conditions thruout the body; the glands, directly

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or indirectly are also modified in their action by the metabolic conditions and by sudden changes of state (as of the emotions) which may be initiated by the environment; gradually under normal conditions and in average individuals these various forces come into an adjustment that represents a person's constitutional norm; in women 13 times a year there is a cyclical interruption of this balance by the introduction of new factors which influence all the endocrines involved and thus upset the balance; in the case of pregnancy, new secretions (and consequent modification of the endocrine balance and of the mental states dependent upon this) are introduced about each of these points: pregnancy, fetal development, parturition, lactation, and the cessation of lactation.

Probably it will be of most service to the readers of this review, in trying to give an idea of the comprehensiveness of the book, to outline the sources, effects and interrelations of the various internal secretions as the author conceives them, especially the more intimate interrelations between the thymus, thyroid, pituitary and the sex glands. The experiments and deductions pointing to, and partially explaining, the cyclical character of these sex-linked endocrine activities seem to the reviewer the most effective and valuable part of the book. The treatment of the sexual and reproductive secretions of the male is not nearly so adequate, in spite of their greater simplicity, as is that of the female.

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Table of Endocrines and Their Actions

(Doubling the signs + and − in column 3 indicates excessive action.)

Source of the Endocrine	Relation to Other Glands	Hyper, + or Hypo, −	Systemic and General Functional Effects	Remarks
Hypophysis:	Associated with thyroid; sex; adrenals.	−	Influences physical, mental and sex development. Leads to dwarfing; sexual infantilism, etc.	Has two somewhat antagonistic hormones. Broadly antagonistic to ovary.
Anterior Lobe		+ ++ − +	Rapid growth at puberty. Normal giants; after puberty, acromegaly. Inhibition of bone development; feminine quality in males. Protective to pregnant uterus; stimulates embryo.	Specially *male* in its influence. With testis, adrenal cortex and interstitial thyroid produces male peculiarities.
Posterior Lobe	Associated particularly with ovaries, uterus, etc. Associated with thyroid insufficiency.	+ +.− + − − ++	Affects metabolism, especially carbohydrate. Trophic control, especially over uterus and genitals. Contributes to libido. Adiposity. Modifies menstruation and accentuates labor. Before puberty, sexual infantilism and inhibition of menstruation; after puberty genital atrophy. Dysmenorrhea; sex feelings more pronounced; nausea, etc., of pregnancy.	Specially *female* in its influence. Coupled with ovary (follicular), adrenal medulla and glandular thyroid, results in peculiarities of the female.
Thyroid	Associated with sex, particularly in female. Mutual hypertrophy of thyroid and parathyroid on removal of either. Excites thymus.	+ − − − ++	Influences calcium metabolism, and activates general growth and function. Retards growth; produces sluggishness and laziness. Myxodaema, cretinism. Excitability, irritability, tremor.	Active at puberty; regresses in old age.
Parathyroids	Antagonistic to thyroids.	+ − ++	Influences calcium metabolism; moderates activity of central nerve cells; and increases sensibility of sympathetic system. Uterine or general tetany in pregnancy. Muscular weakness.	Important in pregnancy. Pregnancy increases their activity.
Adrenals:	Associated with thyroid and hypophysis in sex organs; and development.	+	Antidote for muscular fatigue; protects against toxemia of pregnancy. Aids growth and sex development.	Secretion increased by emotions.
Cortex	Enlarged at pregnancy.	++	More masculine qualities, as courage, anger, muscular energy.	Connected with anger.
Medulla		++	Over-stimulation of medulla, nervousness, anxiety, fear, blushing.	Medulla responds especially to *fear*.
Thymus	Associated with thyroid, parathyroid, pineal, and sex (especially in male). Depressed by ovaries and adrenals	+ + −	Inhibits development of gonads. Thin, short, fragile bones. Hastens sex and other maturity.	Influences calcium metabolism; regress at puberty, and stops inhibiting effect.
Pineal	With thymus; antagonistic to hypophysis.	+ −	Inhibits development of sex glands. Physical and mental precocity.	Develops from 1-7th year.

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SOURCE OF THE ENDOCRINE	RELATION TO OTHER GLANDS	Hyper, + or Hypo, −	SYSTEMIC AND GENERAL FUNCTIONAL EFFECTS	REMARKS
SEX GLANDS:—				
Ovaries (general)	Inhibits (slightly) growth and function of anterior lobe of hypophysis.	+	Tends to control height by effect on epiphyses: late ripening (or castration) increases stature; early ripening interrupts.	
" (special)	Stimulates adrenals and anterior hypophysis.	+ − − + − − +	Trophic control of mammary glands. Castration before puberty prevents libido. Responsible for menstruation. May produce original or regressive infantilism. Protects uterus from too great invasion from placenta.	
Interstitial cells;—(Stroma)	Antagonistic to corpus luteum. Stimulates adrenal cortex and anterior hypophysis.	+ + +	Produce, in connection with other secretions, the secondary female characters at puberty. Control cyclical changes in uterine membrane. Related to formation of blood; dimishes the coagulability of blood. Rhexis; menstruation.	
Follicles (ripening and ruptured);—	Influenced greatly by posterior hypophysis, adrenals, and thyroid. Stimulates adrenal medulla.	+	Trophic to uterus, particularly uterine lining.	These increase old or add *new* secretions to ovary (?) Influenced by and influences menstruation.
Corpus luteum (false; of menstruation):	Reacts on thyroid and posterior hypophysis.	+	Stimulates thyroid in anticipation of pregnancy; inhibits posterior hypophysis to check uterine contractions.	
Corpus luteum (of pregnancy);—	Stimulates glandular thyroid, and inhibits contractile effect of post. hypophysis on uterus.	+ + + +	Inhibits maturation of follicles and ovulation during pregnancy; and sometimes afterward (?) Stimulates growth of decidual cells of lining, and aids imbedding of fertilized ovum. General nutritional effect on uterus in early pregnancy. Protects wall of uterus from too great invasion by trophoblast cells.	Is stimulated by placental secretion of embryo.
Uterine decidua (endometrium)	Mutual stimulation of endometrium and ovary, involving the ovulation-menstruation cycle.	+ −	Sensitizes the ovary. Atrophy of uterus, through inhibition of activity of ovary (follicles).	
Mammary Glands (Nursing)	Hormone antagonizes ovarian activity.	+ + +	Contracts uterus; inhibits menstruation. Atrophy of uterus, and inhibition of ovulation.	Especially where underfunctioning of hypophysis.
Testes:— Interstitial cells. (Other products?)	Operates on, and with, anterior hypophysis, adrenal cortex, pineal, and thyroid.	+ −	To produce general male characteristics of body and mind: heavier and longer bones, shape, hair, changes in pharynx, impulses, etc. Derangement of associated glands, and inhibits sexual growth and differentiation.	Maybe overcome by ovarian extract and thyroid.

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SOURCE OF THE ENDOCRINE	RELATION TO OTHER GLANDS	Hyper, + or Hypo, −	SYSTEMIC AND GENERAL FUNCTIONAL EFFECTS	REMARKS
FETAL (Embryonic):—				
Fertilized egg and Trophoblast:	Inhibits joint work of interstitial secretion and posterior hypophysis.	+	Gives off elements which inhibit menstruation, by checking rupture of blood vessels and escape of corpuscles, even during congestion.	Settles in endometrium, and develops attachments therein.
	Cooperates with corpus luteum.	+	Inhibits normal regression of corpus luteum; i. e., stimulates its growth and activity.	
and Later placenta:	Increases the growth (permanently) and function of anterior hypophysis.	+	Stimulates growth of uterus.	
		+	Protection and trophic effect on both mother and embryo.	
		+	Irritation of mucosa, gastric and other; and nausea.	Counteracted by corpus luteum (?)
Fetus:	Related to placental glands.	+	Plays a rôle in developing function of mammary glands (?)	

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INDEX

A

Abstracts, 14; 89; 158; 190.
Acanthocephala from the Eel, 1.
Ackert, J. E., and Wadley, F. M., Observations on the Distribution and Life History of Cephalobium microbivorum Cobb and of its Host Gryllus assimilis Fabricius, 97.
Allen, W. E., Some Work on Marine Phytoplankton, 177.
Allen, W. E., A Brief Study of the Range of Error in Micro-enumeration, 14.
American Mullets, Life History and Scale Characters, 26.
Animals, Occurrence and Rôle of Copper in, 144.
Animal Parasites, Compendium of Hosts of, 195.
Annual Report of Treasurer, 48.
Arrhenuri, New Species and Collections, 168.

B

Baker, F. C., Preparing Collections of the Mollusca for Exhibition and Study, 31.
Bandler, S. W., The Endocrines, a Review, 200.
Briefer Articles, 14; 89; 158; 187.
Brownian Movement, Microscopical Illumination with Reference to, 158.
Bullard, Chas., A Method for Orienting and Mounting Microscopical Objects in Glycerine, 89.

C

Cambarus agrillicola Faxon, Spring Migration in, 28.
Cephalobium microbivorum Cobb, Distribution and Life History, 97.
Cnidosporidian Spores, Structures Characteristic of, 59.
Combination Lighting, Microscope Illumination with Reference to, 158.
Common Field Cricket, A Sarcophagid Parasite of, 116.
Compendium of Hosts of Animal Parasites, 195.
Copper, Its Occurrence and Rôle in Insects and Other Animals, 144.
Crayfish, Spring Migration in, 28.
Cummins, H., Spring Migration in the Crayfish, Cambarus agrillicola Faxon, 28.
Custodian's Report for the Year 1920, 47.

D

Desmid, Method of Demonstrating Sheath Structure, 94.
Diatoms, Literature of, 187.
Distribution and Life History of Cephalobium microbivorum Cobb, 97.

E

Eel, Acanthocephala from, 1.
Endocrines, Review, 200.

F

Faust, E. C., Larval Flukes from Georgia, 49.
Faust, E. C., Recent Advances in Parasitology, 75.
Fixatives, Effect upon Myxosporidian Spores, 117.
Fresh-water Biology, Ward and Whipple, Compendium of Hosts of Animal Parasites contained in, 195.
Fresh-water and Marine Gymnostominan Infusoria, 118.

G

Galloway, T. W., Review of Endocrines, 200.
Georgia, Larval Flukes from, 49.
Glycerine, Mounting Microscopical Objects in, 89.
Gryllus assimilis Fabricius, Distribution and Life History of, 97.

H

Hausman, L. A., Fresh-water and Marine Gymnostominan Infusoria, 118.
Henderson, W. F., Treasurer, Annual Report, 48.
Herrick, C. A., A Sarcophagid Parasite of the Common Field Cricket, 116.
Hosts of Animal Parasites, A Compendium, 195.
Hubbs, C. L., Remarks on the Life History and Scale Characters of American Mullets, 26.

Digitized by Google

I

Infusoria, Gymnostominan, 118.
Insects, Occurrence and Rôle of Copper in, 144.

K

Kudo, R., On the Effect of Some Fixatives upon Myxosporidian Spores, 161.
Kudo, R., On the Nature of Structures Characteristic of Cnidosporidian Spores, 59.

L

Larval Flukes from Georgia, 49.
Life History and Distribution of Cephalobium microbivorum Cobb, 97.
Literature of the Diatoms, 187.

M

Marine and Fresh-water Gymnostominan Infusoria, 118.
Marine Phytoplankton, 177.
Marshall, Ruth, New Species and Collections of Arrhenuri, 168.
Methods, Department of, 14; 89; 158; 187.
Method of Demonstrating Sheath Structure of a Desmid, 94.
Method for Orienting and Mounting Microscopical Objects in Glycerine, 89.
Micro-enumeration, Range of Error in, 14.
Minutes of Chicago Meeting, 47.
Microscope Illumination, 158.
Mollusca, Preparing Collections for Exhibition and Study, 31.
Mounting in Glycerine, 89.
Muttkowski, R. A., Copper; Its Occurrence and Rôle in Insects and Other Animals, 144.
Myxosporidian Spores, Effect of Fixatives upon, 161.

O

Orienting and Mounting in Glycerine, 89.

P

Pflaum, M., Custodian, Report for the year 1920, 47.
Phytoplankton, Marine, 177.
Preparing Collections of Mollusca, 31.
Proceedings of the Society, 47.

R

Range of Error in Micro-enumeration, 14.
Recent Advances in Parasitology, 75.
Reports of Auditing Committee on Treasurer's and Custodian's Reports, 48.
Reviews, 14; 89;

S

Sarcophagid Parasite, 116.
Scale Characters in Mullets, 25.
Sheath Structure of Desmid, 94.
Silverman, A., Microscope Illinination with reference to Brownian Movement and Combination Lighting, 158.
Spencer-Tolles Fund, Report on, 47.
Summaries, Department of, 75.

T

Taylor, F. B., The Literature of the Diatoms, 187.
Taylor, W. R., A Method of Demonstrating the Sheath Structure of a Desmid, 94.
Treasurer, Annual Report, of, 48.

V

Van Cleave, H. J., Acanthocephala from the Eel, 1.
Van Cleave, H. J., A Compendium of the Hosts of Animal Parasites contained in Ward and Whipple's Fresh-water Biology, 195.

W

Wadley, F. M., and Ackert, J. E., Distribution and Life History of Cephalobium microbivorum, 97.
Ward and Whipple, Compendium of Hosts of Animal Parasites, 195.
Welch, P. S., Secretary, Minutes of Chicago Meeting, 47.

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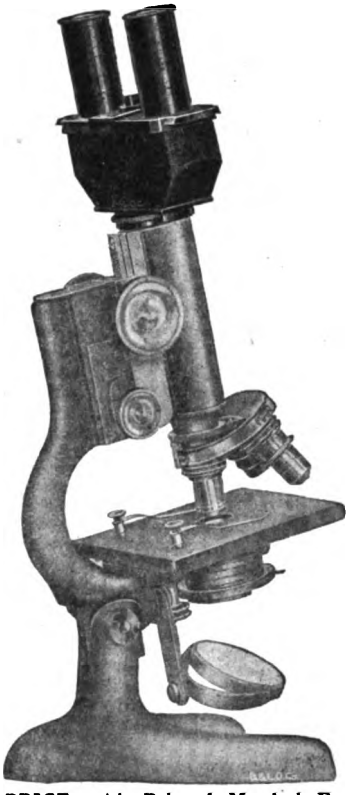

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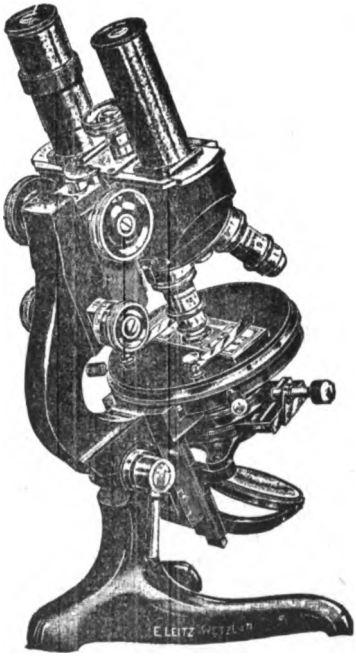

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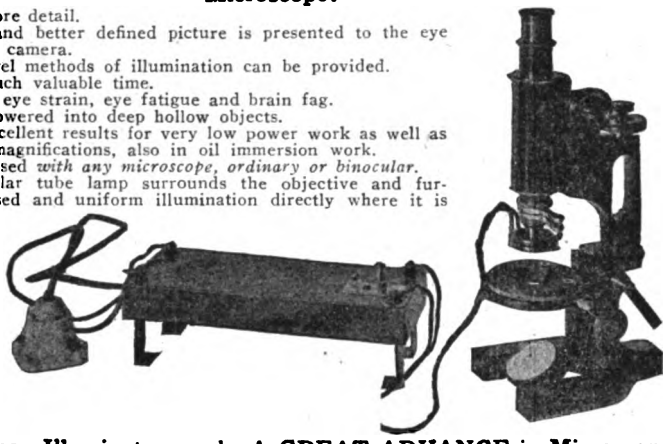

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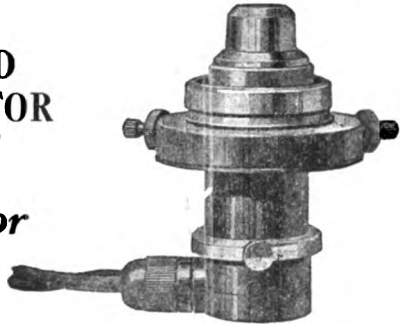

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The Secretary-Editor desires to secure information concerning sets, partial sets, or odd volumes of the TRANSACTIONS which might be secured for the Society either by gift or purchase. Members knowing of such possibilities are earnestly requested to communicate with the Secretary. The earlier volumes are especially needed.

The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the TRANSACTIONS, and of changes of address.

Correspondence relative to membership, publication, exchanges, advertising, etc., should be addressed to the Secretary-Editor.

PAUL S. WELCH,
Secretary-Editor.

University of Michigan,
Ann Arbor, Michigan.

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INDEX TO ADVERTISEMENTS

Anglers Co., Chicago, Ill..... III
Bausch and Lomb Optical Co., Rochester, N. Y..... V
Comstock Publishing Co., Ithaca, N. Y..... III
General Biological Supply House, Chicago, Ill..... II
George Banta Publishing Co., Menasha, Wis..... III
Hubert, H. Edw., New Orleans, La..... II
Kny-Scheerer Corporation of America, New York..... IV
Leitz, E., New York..... VI
Ludwig Hommel & Co., Pittsburg, Pa..... VII
Marine Biological Laboratory, Woods Hole, Mass..... I
Michigan Biological Supply Co., Ann Arbor, Mich..... IV
Powers and Powers, Lincoln, Nebr..... I
Southern Biological Supply Co., New Orleans, La..... I
Spencer Lens Co., Buffalo, N. Y..... II
Sphung, A. A., North Judson, Ind..... VII
Ward's Natural Science Establ., Rochester, N. Y..... III
Watson & Sons, London, Eng..... VIII

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